THE BIOLOGICAL BASIS OF INDIVIDUALITY

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OF
INDIVIDUALITY

A Second Printing

By

LEO LOEB

Professor Emeritus of Pathology
Washington University
School of Medicine
Saint Louis

CHARLES C THOMAS · PUBLISHER

Springfield · Illinois · U.S.A.

Charles C Thomas, Publisher
Bannerstone House
301-327 East Lawrence Avenue, Springfield, Illinois

Published simultaneously in Canada by
The Ryerson Press, Toronto

First Printing, January, 1945
Second Printing, June, 1947

Printed in the United States of America

To My Wife

GEORGIANA SANDS LOEB

Preface

THE STUDY OF individuality began when human beings observed others, noted their structure and functions, their attitudes and actions. At a later stage the physiologist and psychologist recognized that in the individualities separate constituents can be distinguished and they extended the concept of individuality to other organisms than man. In this book the attempt has been made to distinguish between two types of individuality:

The first one is the mosaic type which represents the sum of the particular organ and tissue characteristics (organ and tissue differentials) which determine structure, metabolism, motor and psychical activities and the component parts of which differ in different individuals. These multiple characteristics are combined into a composite or mosaic which is peculiar to each individual.

The second type of individuality which may be designated as the essential individuality is characterized by the presence of a chemical factor—the individuality differential—which is common to the different organs and tissues of each individual and which differs from the corresponding chemical characteristics of the organs and tissues of every other individual. This concept emphasizes the oneness of the individual which depends upon the presence of a common and unique factor in all of his essential parts.

In the same sense in which individuality differentials characterize individuals, there are species, order and class differentials each possessing a specific chemical constitution which characterizes the larger groups of organisms. All these various differentials may be grouped together as organismal differentials in contrast to the organ and tissue differentials which, as mentioned, constitute the mosaic individuality. While it is thus possible to distinguish sharply between these two types of differentials and between the corresponding definitions of individuality, various kinds of interactions take place between the organismal and organ differentials and these interactions are required to make of the individual an integrated whole.

In the following chapters these various aspects of individuality, including the psychical, are analyzed, but only as far as the principles underlying these phenomena are concerned and no attempt has been made to present a detailed or complete account of all the data which may have a bearing on the problems involved.

The starting point of this analysis was a series of investigations on the transplantation of normal and of tumor tissues which the author and his collaborators have carried out in the course of about forty-eight years, some of which, especially those dealing with inbred strains of mice, have not yet been published. To make possible a unified account and interpretation of the various aspects of individuality, it was necessary for one person to undertake this work, rather than to edit a collective book written by specialists in the different

sciences which contribute the data needed for this purpose. The method thus chosen suffers from the difficulty that a single author may not be able to treat with equal competence all the problems involved; but it is believed that the unified presentation of these fields may, to a certain extent, compensate for such a deficiency.

In the following chapters these types of individuality are analyzed as to their evolution and their biological and psychical manifestations.

It is hoped that this presentation may be of interest to the biologist and to the general pathologist and that certain parts of it may be helpful even to the surgeon in the practice of tissue grafting, to the geneticist, to the student of cancer and to the immunologist; perhaps also to the psychologist and to some philosophers.

Acknowledgments

THIS BOOK was written in a provisional form in 1930 and in the following years; however, it was not yet quite finished by 1937. In 1937 a grant was received from the Josiah Macy Jr. Foundation for its completion; this, as well as a revision, was accomplished between the latter date and the present time. The writer wishes to express his appreciation to the Josiah Macy Jr. Foundation for the assistance thus given.

To the International Cancer Foundation the author is indebted for grants which enabled him to undertake additional experiments concerning a comparison between the individuality differentials of normal and cancerous tissues. In this volume the results obtained have been incorporated.

Sincere appreciation is also due to the numerous collaborators who helped to advance this field of research during the many years in which these investigations were carried out. Reference is made in the text to their contributions. The co-operation of Dr. Helen Dean King and Dr. Sewall Wright and subsequently also of others was of very great help in making possible the transplantation of tissues in closely inbred strains of rats and guinea pigs. Later, there were added to these experiments those on closely inbred strains of mice which were received from the New York State Institute for the Study of Malignant Disease, from the Roscoe B. Jackson Laboratory, Bar Harbor, and from other laboratories.

The aid given by the wife of the author, Georgiana Sands Loeb, in the revision of this manuscript, and in other ways through many years, was of the greatest value and to her this book is gratefully dedicated.

To Mr. Charles C Thomas, I wish to express my warm appreciation of the great interest and helpfulness he has manifested in the publication of this book.

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THE BIOLOGICAL BASIS OF INDIVIDUALITY

Introduction

We apply the term *"individual"* to a human being to emphasize the distinctive unique features which such a person possesses. We note his appearance, motor reactions, the expression of his face and his psychical states, especially those which have a social significance. By this designation we accentuate, in general, our impression that the different persons we meet are more or less distinct from one another, although there may be variations in the degree of these differences. Some persons appear to be more like others, while other persons show marked peculiarities which differentiate them sharply from the rest. Attention is given especially to the modes of thinking, feeling, to the emotions, imagination, creativeness, to the behavior in certain social constellations.

We apply the term *"personality"* to a human being, to state our reactions to him in social intercourse, and our opinion as to whether we find him forceful or weak, pleasant or unpleasant, serious or light.

Individuality is here used as the general term, while by *personality* is understood that part of human individuality which manifests and maintains itself in the social intercourse and struggle. We may define this distinction also somewhat differently. Individuality may be conceived of as the original physical and psychical state of an organism, which has developed in accordance with the genetic constitution of this organism with the co-operation of a sequence of more or less fixed physical-chemical environmental conditions. In the course of the natural and social struggle in which a human being is involved, traditions, suggestions, experiences of many kinds, mold this individuality in various directions and thus determine the characteristics which the individual takes on in becoming converted into the personality which develops in the course of time and which alone we know. In this sense we are acquainted not with individualities but only with personalities. The basic individuality is, then, a mere mental construction, which we cannot know but some properties of which we can surmise. However, secondarily, it is customary to express the distinctive features or characteristics of a certain person, which differentiate him from other persons, as his individual characteristics.

These are not sharply defined terms. Like all other beginnings of scientific analysis, they express not yet fully correlated and analyzed experiences; they represent crude approximations to the understanding of reality.

The term "individual" is extended from man to other living organisms which also show distinctive features, and it is applied even to non-living things. In a literal sense, it signifies that an organism or a thing is an integrated whole, which cannot be further divided without ceasing to be this particular organism or thing, without losing its identity. Among the more primitive organisms it may be difficult to distinguish from one another individuals in a given group, but it is possible to differentiate between the larger groups, varieties, species, genera, orders and classes to which the individuals belong.

of one individual recognize different individuals as such, they do more than that, they recognize, to speak in a metaphorical way, the degree of difference between two individuals in accordance with their genetic constitution.

It is not only the cells and tissues of one individual, however, which react towards these elements of another individual in such a specific manner, but there is also a substance in the bodyfluids of one individual which responds towards all the cells and tissues of another individual in accordance with the degree of the genetic difference between these two individuals. This again indicates that there is a constituent common to all the tissues of an organism which interacts with a constituent in the blood serum of another individual.

We may designate this particular characteristic distinguishing one individual from another as his *individuality differential;* it is common to all the various tissues and organs of an individual. In the same way, there are characteristics common to all members of a species, genus, order and class, which may be called species-genus-order-class differentials, and these may be designated in their totality, together with the individuality differentials, as organismal differentials, among which the individuality differential is the highest and finest one. In contrast to these, in particular to the individuality differentials, are the tissues and organ differentials, which differentiate from one another the different tissues and organs, such as liver, kidney, thyroid, cartilage, epidermis, in the same individual.

Theoretically it is of course conceivable that two individuals belonging to the same species, other than unioval twins, possess exactly the same genetic constitution and that accordingly their individuality differentials are identical; but considering the large number of genes which in all probability determine this differential and considering also the possibility that mutations occur spontaneously in the genetic constitution of individuals, such a state of identity must be very rare indeed. Actually it has never been observed in the course of our experiments which were numerous and which extended over a long period of time, except possibly among brothers in a closely inbred family of guinea pigs; but even in this case the actual identity has not been as yet definitely proven. However, as far as the identity of ordinary, non-related individuals of the same species is concerned, the occurrence of such an identity is so improbable that it has not been considered in the chapters of this book, in which only the principles underlying the concept of individuality are discussed.

There are two principal methods by means of which the organismal differentials in general can be analyzed, namely, (1) by various types of transplantation, and (2) by serological methods. As to transplantation, in a wider sense we may include also parabiosis, the joining together of two fully formed organisms and also the uniting of parts of embryos or of blastomeres; even the transfer of a spermatozoon into the egg during the process of fertilization and the joining together of parts of free-living cells, such as protozoa, may be considered as types of transplantation. Transplantation and serological methods are not equally well adapted to the analysis of organismal differentials; each has its own sphere in which it can be applied

Among the higher organisms we distinguish individuals the more readily, the more varied the bodily features and the psychical reactions and the more intimately we are acquainted with the peculiarities which each individual possesses. In the phylogenetically higher organisms the differentiation between the various parts, together with their functions, is greater, and likewise the integration of the parts into one organism is more fixed and rigid. Here it is evident that the individual, as a whole, is the unit in the biological and in the social sense, and not the elements of which the individual is composed—the cells, tissues and organs; nor is a group of individuals, whether a family, clan, nation or race, the real unit. Under natural conditions the smaller component units depend upon the other constituents of the integrated individual for their life and function, but the groups consist of individuals who, if necessary, are able to live and function independently of the other units of the group. It is, therefore, the effects which the actions and policies of the various groups exert on the individual which is the ultimate test of their value. The wellbeing of the group depends upon the wellbeing of the individuals of which it is composed; but conversely, social relationship to other individuals and a healthy group life are conditions which promote the wellbeing of the individual, while unfavorable social relations injure him.

All these individual characteristics in living organisms which we have mentioned so far, are localized in certain areas of the organism, in special organs or tissues, and they are either structural or functional peculiarities of the latter. If we conceive of the individual as *a mosaic of many tissues and organs*, each one functioning and metabolizing in its own peculiar way, we may consider this mosaic of separate parts as the biological basis of individuality, including the psychical characteristics; and in order to understand individuality in this sense we would have to study the peculiarities of the units composing such a mosaic in each individual. Also, the nervous system and the hormone system which serve as means of communication between the various parts of the body, represent special organs or products of organs and are therefore parts of the mosaic. They are the properties of organisms, which are analyzed as to their genetic basis by means of hybridizations according to Mendelian methods.

There is, however, in addition to this mosaic basis of individuality, another basis. There are *properties* which are not restricted to certain parts of the organism, but which are *common to all, or almost all, parts of the organism*, and which, although not visible, bind them together, make them into a unit and differentiate one individual from every other individual; also one species, genus, order, class of organisms from every other species, genus, order and class. There is inherent in every higher individual organism something which differentiates him from every other individual, which can be discovered by observing the reactions of certain cells and tissues belonging to one individual towards the tissues and cells of another individual of the same species. These reactions are indicative of a characteristic common to all the parts of one organism, which differs from the analogous characteristic of all the parts in a different organism of the same species. And not only do the cells and tissues

genesio-, homoio- and heterotransplantation. The transplantation of various kinds of tissues and organ pieces into the same animal from which they were taken and to which, therefore, they belonged, is called *autotransplantation.* Here we find that lymphocytes are practically lacking around the graft; connective tissue cells are attracted in only a moderate number and instead of producing dense fibrous tissue, which is characteristic of their reaction against a strange individuality differential, they form only a loose embryonal stroma around the transplanted cells. The blood vessel supply is rich and in the course of a relatively short time the transplant assumes about the condition of the normal tissue or organ in the host. All tissues from the same organism behave in this respect, in principle, in the same way, except that some tissues can withstand the injury connected with the process of transplantation much better than others. We may then conclude that it is not the organ or tissue differentials which determine these injurious reactions of the host cells towards the grafts, but the individuality differentials. The chemical constitution of liver and of kidney is very different, but this difference has no effect on the host cells—they react in about the same way towards liver and kidney, provided these tissues possess the same individuality differential; however, a slight difference in the chemical constitution of the individuality differential sets unfavorable reactions in motion; and it makes little difference whether the strange individuality differential is attached to organ differentials of kidney, liver, skin, cartilage, uterus or thyroid. The various organ differentials all behave in about the same way.

This, then, is the first important fact: *the host cells recognize in a very subtle way differences in individuality differentials.* But they can do more than this. As stated above, they are able to recognize the degree of difference and to react accordingly. Thus, when a piece of tissue from brother to brother is transplanted—a method designated as *syngenesiotransplantation*—the cells of the one which functions as host are not as much stimulated or excited by the presence of a tissue so closely related to his own as by the tissues from a non-related individual (*homoiotransplantation*), the individuality differentials being more similar in the first case. This observation holds good especially if the parents belong to closely inbred strains; otherwise brothers and sisters may be genetically similar to each other to very different degrees and therefore, in some instances, the reaction against a tissue of a brother may be about the same as against that of a stranger, and if the strangeness exceeds a certain limit, it is no longer the lymphocytes which are active, but the connective tissue cells and the injurious substances of the bodyfluids. On the contrary, in certain inbred strains the individuality differentials of all the animals belonging to such a strain may have become so similar that no or only very slight differences can be established between brothers and not directly related individuals within the same strain.

On the other hand, if a piece of tissue is transplanted from one animal to another which is genetically still further removed than in cases of homoiotransplantation, as when animals from different species serve as host and graft, the reactions are more severe. This procedure is called *heterotrans-*

to the greatest advantage. While the serological tests are especially useful in the analysis of the differentials of groups of animals, such as species, genera, orders and classes, transplantation experiments are best suited for the analysis of the differences between individuals as expressed in their individuality differentials. The study of transplantation among more primitive organisms may contribute to our knowledge of the phylogenetic development of the organismal differentials, and experiments in hybridization as well as in transplantation of embryonal tissues may aid in the analysis of the ontogenetic development of the organismal differentials.

We are concerned principally with the study of that type of organismal differential which we have designated as the *individuality differential,* and here the basic experiment is the following: Various organs or tissues are transplanted from one animal, e.g., a guinea pig, into two other guinea pigs not directly related to the first guinea pig from which the tissues were taken; this is called homoiotransplantation. It is seen that the reactions of the hosts of the multiple grafts toward the latter differ in accordance with the degree of genetic relationship between host and donor, but the host behaves in approximately the same way toward the various tissues from the same donor. In one animal the reactions are severe to all the tissues, in the other one they may be very light. These reactions consist in the activity of the lymphocytes, the connective tissue cells and blood vessels of the host towards the grafts; in addition, tissues, especially the more sensitive ones, are also influenced by the degree of their compatibility with certain constituents of the blood of the host, and the degree of this sensitiveness again depends upon the genetic relationship between host and transplant. In general, tissues are injured by the bodyfluids of a strange host, and in some species this injurious action plays a greater role than in others. However, in all the species which we have studied so far it is *the lymphocytes* which sense or recognize the finest degrees of similarity or difference in the constitution of the individuality differentials between host and transplant. The distinctive reaction of the connective tissue cells becomes noticeable if there is a slightly greater difference between these differentials. The statement that all the tissues from the same donor elicit the same intensity of reaction on the part of the same host is true in a relative, but not in an absolute, sense. Different tissues have an unequal power to call forth these reactions; thus, for instance, thyroid gland usually induces a stronger reaction than cartilage and perichondrium. This is evidently due to the fact that a certain substance responsible for the reaction, the individuality differential, is given off in sufficient quantities more readily by thyroid than by cartilage, which latter has a more inert metabolism. However, notwithstanding these differences between different tissues and organs, in all of them the genetic relationship between host and transplant determines the intensity of the reaction of the host against the individuality differentials of the transplant.

There is a second type of experiment which brings out the meaning of the individuality differential. This introduces variations in the relationship between host and transplant which are expressed by the terms: *auto-, syn-*

genesio-, homoio- and heterotransplantation. The transplantation of various kinds of tissues and organ pieces into the same animal from which they were taken and to which, therefore, they belonged, is called *autotransplantation.* Here we find that lymphocytes are practically lacking around the graft; connective tissue cells are attracted in only a moderate number and instead of producing dense fibrous tissue, which is characteristic of their reaction against a strange individuality differential, they form only a loose embryonal stroma around the transplanted cells. The blood vessel supply is rich and in the course of a relatively short time the transplant assumes about the condition of the normal tissue or organ in the host. All tissues from the same organism behave in this respect, in principle, in the same way, except that some tissues can withstand the injury connected with the process of transplantation much better than others. We may then conclude that it is not the organ or tissue differentials which determine these injurious reactions of the host cells towards the grafts, but the individuality differentials. The chemical constitution of liver and of kidney is very different, but this difference has no effect on the host cells—they react in about the same way towards liver and kidney, provided these tissues possess the same individuality differential; however, a slight difference in the chemical constitution of the individuality differential sets unfavorable reactions in motion; and it makes little difference whether the strange individuality differential is attached to organ differentials of kidney, liver, skin, cartilage, uterus or thyroid. The various organ differentials all behave in about the same way.

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plantation. In this instance the bodyfluids of the host are so different from those to which the tissues of the transplant are adapted that they exert a strongly injurious effect and kill the graft in a relatively short time; the length of time in which this can be accomplished depends, among other factors, upon the degree of resistance of the particular tissue. The reaction of the connective tissue of the host is very strong in heterotransplantation; besides, it is the polymorphonuclear leucocytes which are attracted first, rather than the lymphocytes, indicating the presence of a substance which acts as a stronger poison, a heterotoxin. The reaction of the lymphocytes is the test for the presence of a milder toxin, namely, *homoio-* or *syngenesiotoxin*. However, in places where the toxin action is weaker or at a later period when the acutely-acting toxins have been largely absorbed, lymphocytes may also be attracted and collect in large masses around tissues derived from a strange species. We see, then, that the host cells not only recognize a strange organismal differential, but they also distinguish between *different degrees* of relationship or strangeness. But there is a limit to this power of discrimination. If a certain threshold of strangeness has been reached, the reaction is maximal and cannot be much increased if the tissues from individuals belonging to still further removed classes are used. In this case serological tests are better able to grade differences. The cellular reactions with which we have to deal in transplantation are comparable to a very sensitive balance which indicates small fractions of a milligram and which cannot be used for the detection of differences which are measured by pounds. On the other hand, serological tests are only under very restricted conditions serviceable in the detection of finer differences. Thus, the experiments of Todd (to which we shall refer later) show that under certain circumstances serological tests also may indicate the presence of strange individuality differentials; but only with one particular kind of structure, the erythrocytes, has this test been used, and even then it did not as a rule reveal the degree of relationship or strangeness between the individuals which were compared.

Certain experiments show that the similarity or difference between two individuality differentials corresponds to the similarity or difference in the composition of the gene sets in the host and donor, and that the host cells respond, so to speak, to genes which are strange to them. In reality, however, it is not the genes as such to which the host cells react, but the organismal, and in particular the individuality, differentials which develop in accordance with the gene sets.

That it is the similarity or difference in the gene sets in two individuals which primarily determines the kind of reaction which takes place between host and transplant is also indicated by the fact that if, through close inbreeding, we render their gene composition more similar, the individuality differentials correspondingly become more and more similar in successive generations and the severity of the reaction of the host against the graft is correspondingly diminished. But it has been found very difficult to produce complete identity of the individuality differentials even under these conditions. It seems, moreover, that in different species closely inbred animals differ in respect to the readiness

with which the stage of identity of the individuality differentials is approached or becomes manifest, and the transplantation method can be applied in order to test to what degree the gene composition in the individuals belonging to a closely inbred family or strain has become similar, or, expressed differently, the degree of homozygosity which has been reached in such a strain.

That it is the strange genes in the graft on which the reaction of the host against the transplant depends is confirmed, also, by experiments in which two inbred strains were hybridized and the reactions of individuals belonging to the parent strains against tissues or organs of the hybrids were compared with the reactions of the hybrids against transplants from the parent strains. In the former case the reactions were more severe than in the latter case; this corresponds to the fact that only one-half of the hybrid genes is represented in the inbred parent strains, while the genes of parent strains are all present in the gene sets of the hybrids.

In the course of phylogenetic evolution, gene sets which are characteristic of the more highly differentiated species have gradually evolved from the gene sets of other more primitive ancestor species, and the organismal differentials have undergone a corresponding development. On the other hand, in the fertilized ovum the chromosomes and gene sets are the same as in the cells of the adult organism. Yet there are indications that in the fertilized egg the individuality differential is not yet fully formed, but that it develops from a precursor substance in the course of embryonal life; it is certain that at least the mechanism which makes the differences in the individuality differentials of host and transplant manifest undergoes such an evolution. Even in very young guinea pigs, before the age of sexual maturity, these mechanisms of defense against a strange individuality differential are not yet fully developed, as is indicated by transplantation experiments of tissues into hosts of various ages. The connective tissue reaction is diminished in intensity and the lymphocytes may have therefore a better chance to become active in these young animals.

As to the number of genes which determine the nature of the individuality differentials, no definite statement can be made. However, considering the difficulty in eradicating reactions against other than autotransplants, even in individuals belonging to strains closely inbred through a large number of generations, and considering the improbability of ever obtaining an autogenous reaction after homoiotransplantations in non-inbred strains, also in view of the fact that the reactions are so very finely graded and that a homoio- or syngenesio-reaction after transplantation of a piece of tissue belonging to another individual may appear as late as several months following transplantation, it is very likely that the number of genes entering into the composition of the individuality differential is great and that perhaps all the genes participate, although different ones possibly to a different degree. Both organismal differentials and organ and tissue differentials depend entirely, or to a large extent, on the constitutions of chromosomes and genes; but the genes and combinations of genes which preponderate as determiners of these two types of differentials are evidently not the same and there are indications that it is

certain gene sets rather than individual genes which represent the precursors of organismal differentials. While the individuality differential is therefore determined by the gene composition, it is not identical with the gene sets but differs from them in a way in which other characters of the adult organism differ from the gene sets. Gene hormones may mediate the effects of the genes on the organismal differentials; also, other factors which form part of the environment in which the organism develops may conceivably modify the development of the individuality differential from its precursor substances, and there are indications that adaptive processes which may take place in the interaction between host and transplant may modify these differentials, or at least their manifestation. Such adaptive processes are very prominent in serial transplantations of tumors. Yet there remains constant the difference between the individuality differentials and the organismal differentials in general on the one hand, and the differentials of specific organs and tissues on the other hand; the organismal, and in particular the individuality differentials are the same in all the tissues and organs within the same organism, while each organ and tissue has in addition its specific differential.

There exist, then, perhaps conditions which may complicate the direct relation between organismal differentials and the gene sets which ultimately determine the nature of these differentials. There are, in addition, several other complicating factors. In no case is it possible to determine the organismal, and in particular the individuality differentials directly, but we determine the consequences of the interaction of the organismal differentials of host and transplant; we observe their manifestation and this depends not only on the nature of the organismal differentials but also on the amount of organismal differences produced and given off by the host, and especially also by the transplant, on the degree of reactivity of the host against strange differentials, on the mode of attack on the part of the host, and on the ability of the graft to resist these injurious conditions. A tissue that is readily injured will not give off its individuality differentials for any length of time, because it will be converted into a lifeless foreign body which has lost its specificity.

Notwithstanding these difficulties it is possible to analyze the nature of the organismal differentials if we carry out a number of sufficiently varied experiments, and by these means it is also possible to follow the formation of the organismal differentials in the course of phylogenetic evolution and ontogenetic development, and the gradual refinement of these differentials as indicated by the appearance and increasing significance of the individuality differentials. However, this phylogenetic and ontogenetic development is not represented by a straight ascending line. There are various branches given off by the main line which indicate the development of mechanisms closely resembling the active individuality differentials, but which may not be identical with the latter; in such instances it may not be possible to determine whether we have to deal with real organismal differentials, to which the criteria we have discussed apply. In particular, it is impossible to apply this term in the strict sense to unicellular free-living organisms. Thus the attempt to join together the main body of a rhizopod and a pseudopod, which has

been cut off from either the same individual or from a different individual, succeeds when there is an autogenous relationship between the remaining part of the cell and the pseudopod, but it leads to abnormal reactions when there is a homoiogenous relationship. The nature of the reaction seems to depend on a specific sensitive state of the ectoplasmic layer of the cell protoplasm, and also in part on the diffusion of certain substances into the surrounding medium. In various species of Paramecium peculiar agglutination reactions between different individuals belonging to a certain species have been observed, which are characteristic of each species. In general, cells belonging to the same group do not agglutinate with one another, but individuals belonging to well defined, strange groups of the same species do agglutinate. These reactions resemble those of organismal differentials insofar as relationship between different organisms is a factor which determines the reaction, but they differ from organismal differential reactions in that the reaction seems to depend upon the condition of a restricted portion of the unicellular organism and that specific functions are accomplished by means of these reactions, which are those of certain organs rather than of organismal differentials. A similar problem arises in regard to the relations between spermatozoa and eggs. These relations are in certain respects comparable to those existing between graft and host; but while in the latter an autogenous relationship is most adequate, in the case of sperm and ova a homoiogenous relationship seems in many instances to be as good, or even better, than an autogenous condition. Indeed, in some organisms, plants as well as animals, specific mechanisms exist which tend to prevent autofertilization. These mechanisms depend apparently upon the reaction which takes place, perhaps by means of contact substances, between certain somatic cells belonging to the female organism and the spermatozoa or its analogue in plants, or in other cases they depend upon the direct interaction between egg and spermatozoon.

In the adult organism the various organs and tissues may possess, in addition to the typical species and individuality differentials, structures and substances which are specific not only for this particular organ and tissue, but also for the species to which the organism belongs. The organs and tissues of related species as a rule resemble one another more closely than those of more distant species. The substances which are the bearers of these characteristics may, therefore, have something in common with the species differential or even with the individuality differential substances. However, they differ from the latter in that they are peculiar to a certain organ or tissue. They are not identical with the typical species differentials; this is indicated also by the fact that their chemical reactions may differ in certain respects from those of the typical species or individuality differentials. We may designate these characters and substances as secondary or accessory organismal differentials. In many cases it is not possible to determine to which of these two classes a certain substance belongs and then we must be content to apply the term organismal differential, and in particular, species and individuality differential, in a general way, comprising both the primary and secondary or accessory organismal differentials.

Not every substance produced by tissues or accumulating in certain organs possesses an individuality differential. Many hormones, and the vitamines, do not have individuality differentials, while other hormones have at least some of the coarser organismal differentials. It is especially the most complex protein substances which act as bearers of organismal differentials. But there are end-products of embryonal differentiation in which the cells, which give origin to certain tissues, have been largely replaced by secondary paraplastic substances, such as the lens fibers of the vertebrate eye. In these the finer organismal differentials have apparently disappeared and only some of the coarser ones have remained; instead, the organ differentials have become more prominent. This is indicated if serological tests are used. However, if we use finer tissue reactions as a test, the presence of individuality differentials can be demonstrated even in tissues of this kind, as shown in the recent experiments of H. T. Blumenthal. He has demonstrated that after homoiotransplantation of a lobe of thyroid gland, and of pieces of liver or kidney, from guinea pigs to other non-related guinea pigs, the number of lymphocytes circulating in the blood rises, about five to seven days after transplantation, by approximately 15 to 25 percent, and having reached this maximum it begins to fall again. After transplantation of cartilage however, such a rise is lacking entirely or almost entirely, because the amount of homoiodifferential given off by this tissue is apparently insufficient to reach the threshold necessary for the reaction. After syngenesiotransplantation the increase in lymphocytes begins, on the average, at a later date and remains lower. After heterotransplantation it is the polymorphonuclear leucocytes which show an increase in the general circulation; later they fall to the normal level and this phase is followed by a second phase in which the lymphocytes rise; after a few days this latter rise is likewise followed by a fall. As far as we can judge, these changes in the number and character of the blood cells are specific; inert foreign bodies, for instance agar, do not bring about such a rise. The effects produced by transplants on the lymphocytes and polymorphonuclear leucocytes circulating in the blood are closely parallel to the effects which the transplants exert locally on the lymphocytes and polymorphonuclear leucocytes, but some of the effects of the strange organismal differentials are more readily demonstrated by a study of the cell and tissue reaction taking place around the grafts. By means of this general reaction it can be shown that the lens of the eye also possesses an individuality differential, although, if serological tests are used, it seems to be devoid of species and individuality differentials.

We see, then, that tissues give off substances which differ in their effects in accordance with the genetic relationship of the tissues to the host organism. In their own natural habitat these substances are of an autogenous character and do not incite any abnormal reaction; but in accordance with the genetic strangeness existing between transplant and host, they assume the character of toxic substances, which call forth abnormal reactions in the host. In near relatives these substances—the organismal differentials—act as syngenesiotoxins; in a strange individual of the same species they act as homoiotoxins,

and in a different species, as heterotoxins. The chemical nature of the latter is distinct from that of the syngenesio- and homoiotoxins.

Furthermore, these substances, the organismal differentials, diffuse not only into the area directly surrounding the transplanted piece, but they also enter the circulation and are carried by the blood and lymph to more distant organs. This may be concluded from the observation, already stated, that transplantation of a normal piece of grafted tissue induces changes in the relative proportions of the circulating blood cells, which are parallel to the degree of relationship or strangeness between host and transplant and which depend therefore on the nature of the organismal differentials of host and graft. Such substances, corresponding to individuality and species differentials, enter the blood and exert their effects in distant parts and thus resemble *hormones* in their action.

When they have reached and are retained in certain organs, such as spleen and bone marrow, they may, in addition, stimulate the formation of *immune substances*, since they are strange to the individual or to the species in which they circulate. It is especially the organismal differentials derived from a different species, or even from a different individual, which initiate defensive processes of immunity; being strange to the new host they disturb his equilibrium, which is attuned to substances possessing his own specific organismal differentials. These strange substances act, therefore, as *antigens*. Organ differentials as such may not be strange to the host, in this sense, and as a rule they function as antigens only in combination with a strange organismal differential.

If, then, we may consider it an established fact that when tissues are transplanted from one to another individual of the same species, including even nearly related individuals such as brother or sister, substances are given off by these tissues which call forth noticeable reactions on the part of the host cells, might it not be possible, or even probable, that such substances, acting on nearby tissues as contact substances or on farther distant tissues as hormones, are also given off in the animal's own organism; but that, here, instead of operating as disturbers of the tissue equilibrium, on the contrary, they serve as instruments by means of which the tissue equilibrium is maintained and regulated in such a manner that it is best adapted to the normal cooperation of the various tissues in the interest of the entire organism, and thus to the normal functioning of the organism as a whole? Such substances, representing the individuality differential, if discharged into an animal's own organism may then be designated as autogenous substances. If two tissues, possessing two different individuality differentials, adjoin each other, signs of disharmony develop, which are partly or largely due to the action of disharmonious individuality differentials. This applies, for instance, to homoiogenous skin transplants. Conversely, may we not assume that since the epithelial cells in the normal skin remain at rest, this is due at least partly to the action of the autogenous substances which keep the neighboring epithelial cells as well as the underlying connective tissue and lymphocytes in a quiescent state?

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stimulated; they may assume increased growth and at the same time undergo cerain structural and metabolic alterations. These characteristics may be maintained permanently and when this has occurred, then normal tissues have been changed into cancerous tissues. It can be shown that the latter still possess essentially the organismal differentials of the host from which they are derived; but they differ from the latter by an increase in the growth momentum which enables them in certain cases to overcome, in a new host, injurious conditions to which normal tissues would succumb; they also seem to possess a greater ability to adapt themselves to strange hosts and, moreover, they give off more efficient antigens than do normal tissues.

It is essential for the completeness or fulfillment of the individuality in higher organisms that the integrity of the individuality differentials be maintained. An intrusion of strange substances not bearing the same individuality differential sets in motion reactions which lead to their splitting, their destruction, or their elimination, in some instances after they have been made innocuous through conjugation with other substances. The primary local tissue reactions, as well as the secondary local reactions of allergy and the general reactions of immunity, serve this purpose. But the organism must also build up his species and individuality differentials out of non-specific material or out of material which carries unsuitable organismal differentials; the processes of splitting by means of digestion and those of syntheses lead to the production of building stones endowed with the right type of specificity, and they bring about the replacement of lost tissue and the addition of new material. The *specificity of enzymes* plays an important part in these operations. There are thus strong indications that the individuality differential has these functions: (1) to co-ordinate and to equilibrate the mutual interaction of adjoining and also of some distant tissues in such a way that the inner integrity of the individual is insured, and (2) to combat admixtures from strange organisms and perhaps also to react against foreign bodies which are devoid of organismal differentials.

The organism is, then, a harmonious whole, a combination of the mosaic and of the essential type of individuality; in it, therefore, not only the organ functions are adapted to one another, but also all the various tissues, though apparently functionally unrelated, are specifically adapted to one another, owing to the nature of their organismal differentials. *This latter adaptation is, above all, what characterizes the individual.* Such a harmonious relationship must be based on resemblances or identities in certain chemical structures of the most important and complex substances which enter into the building of the organism, especially substances of a protein nature. Accordingly, it has been established that the hemoglobins and hemocyanins, derived from various species, or from still larger groups of animals, are the most nearly identical in structure in the nearest related animals and are the more dissimilar in structure the farther distant the species are. In accordance with what we have already stated, we may assume that the same chemical gradation in the structure of the organism in correspondence with phylogenetic relationship must go still further, not only each species but each individual possessing its

However, a disequilibrated condition may occasionally be observed even after autotransplantation, for instance, if pigmented skin is transplanted into a defect in white skin of the same guinea pig. Notwithstanding the identity of the individuality differential in this case, the transplanted pigmented epidermis begins to infiltrate the neighboring white epidermis for a considerable time, but ultimately a new tissue equilibrium is established and then the autogenous tissues live harmoniously side by side. The pigmented epithelium is the more active, vigorous tissue, and stimulated by the processes connected with and following transplantation it asserts its superiority over the white epithelium until this stimulation has died out; yet neither connective tissue nor lymphocytes of the host are unduly activated under these conditions, because host and graft possess the same individuality differential.

There exists, then, a mutual adaptation to one another of tissues bearing the same organismal differential, and there exists, also, a mutual adaptation between the blood plasma and the various tissues belonging to the same individual. It is these harmonious interactions which make the unity of the organism possible and which are perhaps the most characteristic feature of the living organism as an individual. But not only are the substances characteristic of each individual different from those characteristic of any other individual and in this sense specific; there is, besides, a second type of specificity, which may be designated as specific adaptation. By specific adaptation we mean that it is the individuality, species, order or class differentials, in general the organismal differentials, attached to the various tissues or to substances derived from these tissues, which determine how suitable and effective the interactions between the tissues and substances are in the performance of certain functions. If the respective organismal differentials are the same in the tissues or substances, the interaction is most perfect. This statement applies, for instance, to the interaction between tissue extracts, blood plasma and blood serum. The character of the organismal differentials attaching to these various substances determines how effective the coagulating power of the extract is, and how effective also the inhibiting action of the blood serum will be.

We may then distinguish *two types of adaptation within the organism.* The *first* one is well recognized; it is represented by the normal interaction of various organs and of parts of organs, and by the transmission of stimuli through the nervous system, through hormones, and through certain other mechanisms. This is the basis of what might be called the mosaic type of individuality. The *second* type is the adaptation which depends on the identity of the individuality differentials of tissues. The integrity of the organ functions is largely based on this identity of the organismal differentials. But in addition a number of chemical interactions in the organism, of which only one example has been mentioned, depend specifically on the character of the organismal differentials which are carried by these substances. This is the basis of what might be called *the essential individuality,* in contrast to the mosaic type.

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stimulated; they may assume increased growth and at the same time undergo cerain structural and metabolic alterations. These characteristics may be maintained permanently and when this has occurred, then normal tissues have been changed into cancerous tissues. It can be shown that the latter still possess essentially the organismal differentials of the host from which they are derived; but they differ from the latter by an increase in the growth momentum which enables them in certain cases to overcome, in a new host, injurious conditions to which normal tissues would succumb; they also seem to possess a greater ability to adapt themselves to strange hosts and, moreover, they give off more efficient antigens than do normal tissues.

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chemical characteristics, which differ from those possessed by every other individual of the same species.

Two possible schematic representations of the chemical constitutions of two different individuals may be considered: (1) Individual A: T_{1a}—T_{2a}—T_{3a}—T_{4a}. . . . Individual B: T_{1b}—T_{2b}—T_{3b}—T_{4b}. (2) Individual A: T_{1a}—T_{2b}—T_{3c}—T_{4d}. . . . Individual B: T_{1m}—T_{2n}—T_{3o}—T_{4p}. T_1, T_2, T_3, T_4 represent organ and tissue differentials, such as those of liver, kidney, thyroid, cartilage and ear. Provisionally they may be assumed to be identical in two individuals belonging to the same species and variety, although this assumption may not be entirely correct. a, b, c, d represent the individuality differentials which are different in the corresponding organs and tissues of two individuals. In the first mode of representation all the organs and tissues of individual A have the factor a in common, while in individual B all organs and tissues have the factor b in common; a is the individuality differential of individual A; b is the individuality differential of individual B. In the second mode of representation each organ and tissue of individual A has its own specific factor; one has a, the other b, a third one c, and so on, while the organs and tissues of individual B also are distinguished from one another by specific factors, m, n, o and p . . . but the factors in individual A differ from those of individual B; the factors a, b, c and d . . . in their totality represent the individuality differential of individual A, while the factors m, n, o and p . . . in their totality represent the individuality differential of individual B. According to the second mode of representation each organ and tissue differential of an individual possesses its own index of individuality, and every organ and tissue would possess a secondary or accessory individuality differential. According to the first mode of representation the individuality differential attached to each organ and tissue of individual A would be the same, and those attached to the individuality differentials of the organs and tissues of individual B would be identical; these individuality differentials would correspond to the primary individuality differentials.

It will be necessary to decide between these two possibilities. If we adopt the first mode of representation, the various tissue constituents, lymphocytes, fibroblasts, blood vessels, polymorphonuclear leucocytes, would react against all the constituent parts of individual A in about the same manner, because these parts have the same factor in common. The same applies to constituent parts of individual B. But if we adopt the second interpretation, each of these tissue constituents would have to remember—to speak metaphorically—an endless number of tissue and organ factors which are attached to the constituents of its own body and would have to distinguish these from a multiplicity of tissue and organ factors possessed by a different individual. Correspondingly, the blood serum of individual A would be favorable to all the constituent parts of individual A, and would be less favorable to all the constituent parts of individual B, because the factor a, or a factor correlated with a, in the blood serum of individual A would be adapted to the tissue and organ factor a of individual A, and would be less favorable to factor b of individual B. The second concept would only with great difficulty explain these specific

reactions between the tissue constituents and the blood serum of the host and the transplant. We may then conclude that each organ and tissue of individual A has in common a chemical factor which differs from the corresponding factor in individual B; but in addition, certain organs and tissues may possess accessory or secondary individuality differentials, which are peculiar to these organs and tissues.

The individuals among the higher organisms possess, then, two kinds of adaptive mechanisms: the first one is that represented by the functioning and interaction of tissues and organs within the individual, and the second is based on the fact that the tissues and organs in the same organism possess the same individuality and species differential, and that other individuals or species carry different organismal differentials which are graded according to the phylogenetic relationship. In consequence of this functional and structural constitution, very specific relationships have developed within the individual organism and between the various inidviduals within the same species, genus, order and class. These intricate and complex specificities of both the mosaic and essential type in their totality constitute the characteristic feature of the individual.

However, within the functioning organism, as well as in the relations between different individuals, the organ and tissue specificities are more obvious than the individuality differentials, the effects of which are of a more subtle nature. Also, in the sphere of social-psychical relations it is the function of organs, above all, the nervous system and the endocrines, which appears as the significant element. Yet, the individual organism is an integrated whole and changes in one organ and tissue are followed as a rule by changes in other organs and tissues. This applies also to those organ and tissue modifications which occur during the process of ageing and disease, and also to interactions between organs which concern primarily vegetative functions, as well as those which control the psychical-social activities, and both of these two latter kinds of processes are linked together. Every change in a part of an individual affects the individual as a whole, although different types of interferences may differ in their effects on other parts of the organism and on the individual as a whole.

Individuality, especially its social-psychical aspect, entered the experience of man in very early periods of history; it helped to shape tradition and was one of its important components, and as such it took part in orienting the rules of conduct and of law. Gradually, under its influence, philosophical systems and various metaphysical concepts arose. But it was only during the 19th. century that the concept of individuality was fully dissociated from its practical social implications and that it began to be considered *a biologicol problem;* from then on observations and experiments in various fields of biology have contributed to its analysis and more definite problems concerning the individual and individuality were formulated. We shall here record a few of the principal contributions concerning the biological aspect of individuality.

The botanist, Naegeli, conceived of a substance peculiar to each individual or species and he distinguished it from other less important constituents of the living matter as the *idioplasm.* It served as the carrier of the characteristics

which were inheritable and transmitted from generation to generation. The botanist, Strassburger, and the zoologist, O. Hertwig, localized this hereditary substance in the *nucleus*. Subsequently, based on the work of Mendel and of those who rediscovered and continued his investigations, the idioplasm became more sharply defined and transformed into sets of discrete units, the *genes*, which are contained in the female and the male germ cells, and especially in the chromosomes of the cell nucleus. But in addition to these genes located in the chromosomes, also constituents of the egg cytoplasm were considered as determiners of the distinctive features of the organism, and some geneticists (Correns, v. Wettstein and Kühn) have assumed that genes, equivalant to those situated in the chromosomes, may also exist in the cytoplasm of the egg.

During the second half of the last century certain observations of surgeons, who grafted tissues, pointed to a fargoing specificity and individualization in the tissues comprising the higher organisms. The early experiments of the French surgeon, Ollier, showed that only autotransplanted bone was able to survive. Later experiments with skin and various other organs or tissues, such as ovaries, and also with benign tumors, indicated that while homoiotransplantation might perhaps succeed, autotransplantation was more favorable. Yet, towards the end of the last century biologists found that the joining together of parts of embryos, as well as various kinds of homoiotransplantations of invertebrate and lower vertebrate tissues, may give good results, and that even heterotransplantations were successful under certain conditions. Notwithstanding the experiences mentioned above, in the transplantation of tissues in adult mammals no sharp distinction, as a rule, was made between the results of auto- and homoiotransplantation. This was true even of the work of Reverdin and Thiersch, who introduced skin-grafting into surgery for therapeutic purposes. While, as stated, some of these observations suggested that biochemical differences might exist between different individuals, including those belonging to the same species, on the whole, the differences between species were stressed rather than the differences between individuals, and it was only within the last thirty-five or forty years that *the distinctions existing between the tissues of different individuals of the same species received more attention.*

In the meantime, discoveries in biochemistry, comparative anatomy and embryology had led to a further analysis of the specific structure of organisms. The biochemist, Huppert, in 1895, in a lecture on the persistence of species characters, referred also to the differences which, according to Rollett, existed between the hemoglobins of different species as regards their elementary composition, solubility and shape of crystals. He concluded that not only the chemical constitution of various substances differs in different species, but also that the metabolism of these substances is distinct and characteristic of these species. Four years later Rabl, apparently under the influence of Huppert's suggestions, discussed the differences in the microscopic structure of homologous tissues in different species. While the histologic structure of the liver could not be distinguished, it was discovered that the lens of the eye differed in different species. This difference was maintained during the various

stages of development and also throughout life. Furthermore, the organ-forming, germinal areas of His, and the cells which compose them, were observed to be specific in their form and in the possession of organ-specific substances, and His traced this specificity back to the germ layers, to the blastula, and, in the end, to the different parts of the unsegmented egg. It is this specificity to which he attributed the differences in the embryonal development of different species. Accordingly, Rabl and, in particular, Conklin were able to follow the development of organs from the egg through the first segmentations and through later stages to the complete organism; protoplasmic movements and the character of mitoses were found to correspond to the specific structure of the species. Rabl concluded that the specific characteristics of the organism, or rather of the species, as a whole, determine the specific features of all the organism's component parts—its organs, tissues and individual cells. In the discussion of this investigator we find, therefore, already a suggestion that besides the differences in the organs and tissues which distinguish different parts of the individuals as a species, and even different parts in the unsegmented ova, there is something in the species as such which determines its characteristic development in both the structural and chemical aspects. This species peculiarity became manifest also in transplantations; homoiotransplantations as a rule succeed, while heterotransplantations are unsuccessful. It is evident also in blood transfusions, which may be considered as modified transplantations.

Oscar Hertwig named the factor which made homoiotransplantation possible, but which caused incompatibility of heterogenous parts of organisms, "vegetative" affinity, and contrasted it with "sexual" affinity which was responsible for successful fertilization. He believed that in plants as well as in animals vegetative and sexual affinity are similar in their manifestation and are due to the same underlying factors. These suggestions of Hertwig were taken up later by W. Schultz, when he tried to prove the parallelism between hybridizability and transplantability in the tissues of vertebrates. However, as we shall see later, while a certain parallelism is noticeable between these two processes, there are also some marked differences.

It is noteworthy that the concepts of Naegeli and Hertwig related mainly to species, not to individuals. In the meantime, towards the end of the last century, the development of the new science of immunology had set in, but it was likewise primarily concerned with species differences, and only secondarily and somewhat later, with differences between individuals. But the investigators in the field of transplantation and immunity influenced, also, some biologists, as is noticeable in the writing of Fick, who in 1907 added to the concept of the species plasma that of an individual plasma. The fertilized egg of one individual was assumed to differ from those of all other individuals of a certain species in regard to the character of its organ-forming substances; however, a distinction was made between the living protoplasm, in which these specificities applied, and the trophoplasm, which represents merely food and structural material, and which was less specific or nonspecific. It was recognized that the living protoplasm consists essentially of protein. Each

which were inheritable and transmitted from generation to generation. The botanist, Strassburger, and the zoologist, O. Hertwig, localized this hereditary substance in the *nucleus*. Subsequently, based on the work of Mendel and of those who rediscovered and continued his investigations, the idioplasm became more sharply defined and transformed into sets of discrete units, the *genes*, which are contained in the female and the male germ cells, and especially in the chromosomes of the cell nucleus. But in addition to these genes located in the chromosomes, also constituents of the egg cytoplasm were considered as determiners of the distinctive features of the organism, and some geneticists (Correns, v. Wettstein and Kühn) have assumed that genes, equivalant to those situated in the chromosomes, may also exist in the cytoplasm of the egg.

During the second half of the last century certain observations of surgeons, who grafted tissues, pointed to a fargoing specificity and individualization in the tissues comprising the higher organisms. The early experiments of the French surgeon, Ollier, showed that only autotransplanted bone was able to survive. Later experiments with skin and various other organs or tissues, such as ovaries, and also with benign tumors, indicated that while homoiotransplantation might perhaps succeed, autotransplantation was more favorable. Yet, towards the end of the last century biologists found that the joining together of parts of embryos, as well as various kinds of homoiotransplantations of invertebrate and lower vertebrate tissues, may give good results, and that even heterotransplantations were successful under certain conditions. Notwithstanding the experiences mentioned above, in the transplantation of tissues in adult mammals no sharp distinction, as a rule, was made between the results of auto- and homoiotransplantation. This was true even of the work of Reverdin and Thiersch, who introduced skin-grafting into surgery for therapeutic purposes. While, as stated, some of these observations suggested that biochemical differences might exist between different individuals, including those belonging to the same species, on the whole, the differences between species were stressed rather than the differences between individuals, and it was only within the last thirty-five or forty years that *the distinctions existing between the tissues of different individuals of the same species received more attention.*

In the meantime, discoveries in biochemistry, comparative anatomy and embryology had led to a further analysis of the specific structure of organisms. The biochemist, Huppert, in 1895, in a lecture on the persistence of species characters, referred also to the differences which, according to Rollett, existed between the hemoglobins of different species as regards their elementary composition, solubility and shape of crystals. He concluded that not only the chemical constitution of various substances differs in different species, but also that the metabolism of these substances is distinct and characteristic of these species. Four years later Rabl, apparently under the influence of Huppert's suggestions, discussed the differences in the microscopic structure of homologous tissues in different species. While the histologic structure of the liver could not be distinguished, it was discovered that the lens of the eye differed in different species. This difference was maintained during the various

To return to the individuality in the structure and chemical constitution of the egg, the conceptions of Fick and others evidently do not localize these characteristics in the nuclear genes, but in the cytoplasm. There can be no doubt at the present time as to the significance of the chromosomes and genes, or other subdivisions of chromosomes, and of the arrangement of the latter in the chromosomes, for the determination of species and individual characters although differences of interpretation exist as to the mode of their representation in the chromosome. There is further no agreement, as yet, among investigators as to the importance which must be attached to other factors in addition to the chromosomes. We have referred already to the views of Correns, von Wettstein and Kühn, who assume that also the cytoplasm carries genes which determine development; this conclusion was based on the results of species hybridizations in which reciprocal combinations gave different results and in which an influence of the maternal germ cells was noticeable, in accordance with the views of Jacques Loeb (1916), who had restricted the Mendelian mode of heredity to certain individual characteristcs of organs and tissues, while he believed that species characteristics are determined by the cytoplasmic structure of the ovum. In a similar way von Wettstein assumes that the hereditary substance which is localized in plasma differs in its significance from that of the genes of the chromosomes. He suggests that it is the former which is the real substratum of the developmental processes, whereas chromosomal genes and environmental factors control and direct the processes which are dependent upon the structure of the cytoplasm.

However, the majority of geneticists at the present time hold that the genes are the substratum which determines the hereditary transmission not only of the individual but also of the species, genus and class characteristics from generation to generation, and that the genes impress upon the cytoplasm of the ovum, by their interaction with the latter, the structure which is specific for each species. We would, accordingly, have to assume that the individual, species, genus, order and class differentials, in general the organismal differentials, are preformed in both egg and spermatozoa and that, thus, precursors of the organismal differentials exist in these germ cells, the nature of which is determined by the genes of the egg and spermatozoon. However, whether all the genes participate equally in the determination of the organismal differentials, or whether some of the genes predominate in this function over others, is not known. Inasmuch as no difference has been found between the male and female sex in regard to the transmission and possession of the organismal differentials or their precursors, it may be assumed that the Y chromosome, which is concerned with the sex differentiation of the fertilized egg, does not play an essential role in the determination of the constitution of these precursors. This would accord with the indications which exist that the Y chromosome does not as a rule carry demonstrable alleles of sexlinked genes, at least not many of them. This interpretation of the nature of the precursors of the organismal differentials is the most probable one, because egg and spermatozoon, as far as we know, contribute equally to the constitution of these precursors. In regard to the organ and tissue differentials, however, which characterize and distinguish the various organs and tissues in the same indi-

organ-forming substance has individual peculiarities which depend upon the presence of special chemical groups or on stereoisomeric groups in these proteins, but it was not considered probable that this specificity was based on the existence of giant protein molecules, which Pflüger, and later Verworn, identified with the living protoplasm. It may be added that Herbst subsequently attributed individual differences to sidechains or smaller radicles of these complex substances rather than to the characteristic structure of giant protein molecules as a whole. However, Fick did not interpret the individual specificity of an egg as due to a single specific substance, but to the sum of peculiarities in the different organ-forming substances in the egg, which latter had been postulated as early as 1880 by the botanist Sachs. Similarly, the individual plasmas of the spermatozoon and of the unfertilized ovum were held to be united in the fertilized ovum, but this combination was thought to lead not merely to a summation of the properties of these plasmas, but to a new specificity. The individuality of the egg represented thus a mosaic of individual peculiarities in the organ-forming substances, and the individual plasma of Fick is therefore quite distinct from the concept of the individuality differential. In the first place it refers to the constitution of the egg and not to the differentiated and integrated individual, and, insofar, it might correspond to precursor substances of the individuality differentials. But it differs from the concept of the individuality differential in that it represents the peculiarities of the mosaic of organs and tissues, or of their precursors, the organ-forming substances of the individual rather than those of a substance which is common to all of these organs and tissues. Subsequently Correns restricted the meaning of the "individual plasma" of Fick and substituted for it the specific plasma of pure lines in the sense of Johannsen. However, it is clear that in the higher organisms which propagate by fortuitous cross-fertilization such pure lines do not exist and transplantation experiments indicate that among the higher classes of animals pieces of skin of different individuals differ from one another. As far as we know now, the results of homoiotransplantation are never quite the same as those of autotransplantation.

We see, then, that Fick's concept of "individual" referred to organ specificity, to inherited peculiarities of organs or their differentials. This appears also to be the concept of G. Jaeger, who many years previously had postulated differences in the chemical constitution of individuals belonging to the same species on the basis of differences in scents, which may serve to distinguish individuals and species. This kind of specificity concerns individual differences in certain tissues and not something which is the same in all the tissues of an individual.

Likewise the term "homology," as used by the comparative anatomist Gegenbaur, expresses the similarities in the phylogenetic evolution of corresponding organ systems. Both comparative anatomy and paleontology consider the similarity inherent in the organs in different species as an indicator of their phylogenetic relationship, and they trace evolution by means of these homologies found in fossils and in still existing species. In these instances we have again to deal with organ specificities and therefore with something distinct from the organismal differentials.

acter. The tissues of the intestines and various other organs then build up from these split products new complex proteins which possess the specific species character of the new host. Hamburger (1903) assumed that special chemical groups characterize the different proteins of a certain species and that these groups are common to the various tissues of the same organism. Similar were the conceptions of Obermayer and Pick, who at this time had begun their investigations into the chemical factors which determine the species-specificity of proteins. Hamburger extended these ideas also to individuals and he held that the proteins of each individual have a chemical characteristic in common, which differs from that of every other individual belonging to the same species. Subsequently it was found that in addition to the proteins, also carbohydrates and lipoids, as well as other simpler substances, if they are combined with foreign proteins (Landsteiner) can serve as antigens which give rise to specific immune bodies, and that these non-protein substances as such may interact in a specific way with these antibodies. It had thus become possible to differentiate by means of immune reactions between substances characteristic of different species and thus to obtain tests for species differentials; also, in a few cases, to differentiate by these means even between different individuals belonging to the same species (Ehrlich and Morgenroth, Todd).

Furthermore, a distinction can be made in this way, not only between species differentials but also between the characteristic constituents of different organs and tissues within the same organism (organ differentials); and it was found that, as a rule, it is necessary to combine the organ differentials of one species with a strange species differential in order to produce organ-specific, immune substances; these combined antigens are an expression of the intimate relations which exist also embryologically and genetically between these two types of differentials or their precursors. In certain protein substances both species-specific and organ-, tissue-, or substance-specific groups may be present.

It has been mentioned above that observations made in the grafting of tissues led to the conclusion that constitutional differences may exist between individuals of the same species; we have referred already to the work of Ollier, who in the second half of the last century found differences between the readiness with which periosteum of one animal can be transplanted to the same individual and to other individuals. Similar differences were noted also in the transplantation of other organs, above all, in the *skin-grafting* which Thiersch perfected; according to Schoene, Thiersch had suggested biochemical characteristics as the cause of these differences. Somewhat later, Knauer found that also in the case of *ovaries*, autotransplantation was more successful than homoiotransplantation. Between 1901 and 1907 we carried out, in rats and dogs, several series of transplantations of mammary gland adenoma, which were intermediate in character between normal and cancerous tissues, and found that while autotransplanted pieces continued to live and in some cases proliferated, homoiotransplanted pieces died.

We recognized three factors as responsible for this result: (1) the exist-

vidual, species, and so on, these depend in all probability on the structure of the egg and on organ-forming substances which are distributed in a definite and characteristic manner in the egg of each species, and on the interaction of the organ-forming substance with the nuclear genes, in accordance with the fact that genes have a specific relation to organ peculiarities of individuals or species.

There exist, then, in the fertilized egg not only the precursors of the organismal differentials but also those of the various organ and tissue differentials, which latter, singly or in their totality, likewise characterize an individual or species and which constitute the mosaic parts which have served in the study of Mendelian heredity.

Comparative anatomy, embryology, genetics and biochemistry have thus contributed to the analysis of what may now be designated as organismal and, in particular, as individuality differentials on the one hand, and organ and tissue differentials on the other hand. Furthermore, at about the time when biochemists, anatomists and embryologists began to discuss the problem of the chemical and structural basis of species differences, an important additional stimulus to the analysis of this problem was given and new viewpoints were revealed by the development of *immunology.* Following the discoveries of Pasteur, Behring, Roux, Buchner, Ehrlich, Metchnikoff, Gruber, Kraus and others concerning the production of an active and passive immunity against microorganisms and their toxins, and the mechanisms underlying this immunity, it was found by Bordet, Tschistowitch and other investigators, that similar immune reactions can be called forth against bodyfluids and cells of organisms belonging to different species. As a result of this immunization various kinds of antibodies, such as *precipitins, agglutinins, hemolysins* and *complement fixing substances* are produced, corresponding to the antibacterial and antitoxic substances which had previously been discovered. These findings suggested the possibility of differentiating different species by means of such antibodies. If blood sera or other substances of a protein nature from various species were injected repeatedly into rabbits or other animals, immune sera were obtained against the antigens used. The interactions of these immune sera with the antigen and with other analogous substances obtained from nearly related or more distant species were then compared and the results of these tests served as indicators of the relationship between the various species (Grünbaum, Nuttall).

In a similar way the interaction of performed bodyfluids and cells derived from different species was tested directly (Friedenthal). Landsteiner (1901) studied the interaction of individual human sera and erythrocytes in order to find differences between different individuals; instead, he discovered the existence of *four different blood groups* into which human beings can be arranged. Hamburger (1901), and later, Abderhalden, pointed out that proteins derived from a foreign species are toxic if introduced parenterally and that it is the function of the gastrointestinal organs to split these proteins into simpler constituents, which are no longer characteristic of the species from which they were derived and which at the same time have lost much of their toxic char-

individuality, of the contrast between mosaic and essential individuality, of the phylogenetic and ontogenetic development of the organismal and, in particular, of the individuality differentials which we have mentioned in the first part of this chapter, and which will be discussed in greater detail in the following chapters.

At first various fields of investigations relating to the biological basis of individuality, which have been enumerated in the preceding discussion, developed separately, but gradually an interaction between these diverse lines of investigations was established and proved fertile. In the beginning of this century it was mainly the concepts of immunology which greatly influenced the study of the transplantation of tissues, but later a reciprocal influence became noticeable and during the last fifteen years the analysis of individuality by the method of transplantation has stimulated also the search for individuality-differentials in various antigens by immunological methods.

In the studies mentioned so far, the question of individuality and specificity was considered from purely theoretical points of view. But the requirements of social life and especially also the need to sustain the health of body and mind of the individual members of a community and the harmonious relations between those that compose a social group, made it necessary, to face the problem of individuality from a somewhat different viewpoint. This has led to the concept of "constitution" as something which is peculiar to individuals and allows the classification of certain groups of individuals according to their reactions to various environmental conditions. It was observed that different individuals behaved differently in the same environment and under apparently identical conditions. On this basis a distinction was made between the environmental factors and the substratum on which the latter act. The mode in which the substratum responded to conditions in the outer and also in the inner environment, depended on and revealed the constitution of this substratum. While various characteristic features of a constitutional nature were shared by a number of individuals, in the totality of these features each individual was unique and differed from every other one; and it was especially the physician for whom these individual- and group-constitutional differences were of practical importance. Thus the concept "constitution" developed in response to the needs of daily life, and it accentuated, as does also the concept "individual," the contrast between the organism and the outer world. The *analysis of constitution* is therefore another step in the delineation of individuality and personality from the surrounding world. It is an attempt to determine what in our interaction with the living and non-living world around us is due to ourselves and what is due to the world outside ourselves. But here great difficulties arise because of the great complexity which exists in the interaction between the individual and the outer world, and between the outer and inner milieu of a higher organism.

However, through experiment and observation it has been learned that certain characteristics of an organism are fixed in the germ cells and give rise to certain structural, metabolic and functional conditions in the individual. These inherited features represent the core of his constitution; it is the unchangeable part of it. But in actual life it is often very difficult or impossible

ence of certain conditions in the bodyfluids of the host, which determine the suitability of the animal's own autogenous bodyfluids and the unsuitability of homoiogenous bodyfluids for the transplant. This conception implies a factor in common to the bodyfluids and to the cells of each individual. In subsequent investigations to which we have referred already also Todd (1913) recognized the existence of a factor common to and characteristic of all the cells of an individual; but according to this investigator in near relatives this factor might be the same. (2) Growth factors inherent in the transplant, and (3) extraneous growth factors circulating in the bodyfluids of the host, similar to those given off by the ovary under certain conditions. These observations were subsequently confirmed by Borrel, Ribbert, and they were extended to malignant tumors by Bashford and Tyzzer. In 1909, Borst and Enderlen referred the difference between auto- and homoiotransplantation of blood vessels to "biochemical differences" between individuals of the same species. However, as we shall show later, these "biochemical differences" are not identical with individuality and species differentials. With Addison, we extended our investigations as to the effect of the phylogenetic relationship of tissues belonging to different species on the fate of the transplants, and Schoene studied the influence of family relationship on transplantability. In the following years, W. Schultz analyzed the relation between hybridizability and transplantability of skin. In the meantime tumor transplantations had been carried out on a large scale, and while at first, especially in the work of Jensen and Ehrlich, problems of immunity played a prominent role in the analysis of conditions which determine the result of transplantations, we and, subsequently, Peyton Rous used the *transplantation of tumors as a method for studying tissue growth* in general and we emphasized the close relations which exist between the growth of normal tissues and of tumors.

The writer, in association with Addison, Myers, Hesselberg and others, noted the significance of lymphocytes, and also of fibroblasts and vascular endothelia, in the reaction of the host against the transplant. In the case of tumors, it is especially the various investigations of Murphy and his collaborators which subsequently showed the significance of lymphocytes in the resistance of animals against inoculated pieces of cancers. In normal tissues we found that the time of appearance and the intensity of the lymphocytic reaction developing around a transplant in combination with connective-tissue and blood-vessel reactions, could be used in testing quantitatively the genetic relationship between host and donor. We formulated thus, in the following years, the concept of *organismal differentials,* and we analyzed the genetic relationship between host and transplant and the fate of the latter. Closely inbred strains of mice were used for the analysis of the factors determining the growth of tra[illegible] and we extended [illegible]tials of normal tissues. There developed hence, step by step, mainly as the result of greatly varied transplantations of normal tissues in which simultaneous multiple and successive transplantations proved of special value, the conceptions of various kinds of specificity in tissue and organ relations, of the autogenous equilibrium of the organism and of tissue reactions as a test for

Part I

Transplantation of Tissues in Higher Vertebrates as a Method for the Analysis of the Organismal Differentials

Chapter 1

General Considerations

OUR ANALYSIS of individuality and organismal differentials is based primarily on investigations into the fate of transplants of normal tissues, and also of tumors, and their genetic relationship to the host in which they live. In such an analysis it will therefore be necessary to discuss in more detail the results we have obtained in these experiments, especially in the ones on which a full report has not as yet been published. However, before entering into a discussion of these results, the following questions, which relate to these investigations in general, will be considered: (1) the aim of these investigations; (2) the factors which have to be taken into account in evaluating the conclusions, namely, a) the mode of interaction between host and transplant and the various reactions of the host which are induced by the transplant, b) the differences existing between different species, c) the differences existing between different strains and individuals, d) the differences existing between different tissues serving as transplants; (3) the methods which best serve our purposes and the variable factors which may complicate the application of these methods, and (4) the possible errors which have to be considered in these experiments.

1. *The aim of these investigations* is the analysis of the organismal differentials of individuals, families, strains and species. We are not primarily concerned with various other problems, as, for instance, the conditions under which tissues survive and the establishment of the methods most suitable to accomplish their survival; the analysis of polarity in the structure of various organisms and tissues, and the question of the factors which determine the growth of the grafts or the fate of pigmented tissues. Only in so far as such problems aid in the analysis of the organismal differentials and, in particular, of the individuality differentials, are they to be considered. But these investigations, in addition to their primary objective, contribute also secondarily to our knowledge of tissue reactions in general, of factors which are active in pigment formation, and to our understanding of the potential immortality of tissues. In a wider sense, our interest centers in the phylogenetic and ontogenetic evolution of the organismal differentials, in the relation of these differentials to organ and tissue differentials, and to the psychical differentiation and the social life of individuals.

2. (a) *As to the mode of interaction between host and transplant,* the following factors have to be considered: (1) The effect which the bodyfluids of the host exert on the transplanted tissues; (2) the effect which the connective tissue and blood vessels of the host have on the state of the graft; (3) the

to separate this core from effects produced by the environment. Therefore the physician especially is forced to adopt as the definition of constitution not only the genotype, the inherited part of the individual, but also those effects of the environment which have modified his mode of response to environmental factors in a more permanent way. But different environmental factors differ greatly in their intensity and in the perpetuity of the constitutional changes which they produce and in the number and imporance of the parts of the organism which they affect. All transitions exist in this respect and no sharp line of demarcation can be found between various environmental factors. It is particularly the *nervous system* which responds most readily to the environment in the mentally most plastic organism, man. Every thought and suggestion which he has received produce an important change in his constellation as far as his behavior is concerned. Constitution thus becomes identical with the constellation of an organism produced by all kinds of inner and outer conditions, which regulate future reactions; it depends upon the condition of organs or organ systems and corresponds therefore to the mosaic type of individuality. But the term "constitution" has received a different content under different circumstances; it is not sharply defined, yet it may serve as a provisional instrument in the analysis of the reactions of an individual.

In the following chapters we shall discuss more fully the different aspects of individuality, to which we have referred in this introductory review. In the various parts of this book the following problems will be discussed:

Part I. The transplantation of tissues in higher organisms which furnishes the most delicate tests of individuality differentials and is the basis on which further theoretical considerations have been built.

Part II. The phylogenetic and ontogenetic development of individuality and organismal differentials, from the primitive to the highest organisms and from the egg to the adult state.

Part III. Conditions suggesting or simulating the presence of individuality differentials which exist in certain unicellular organisms, either free-living, such as certain protozoa; or representing parts of more complex organisms and constituents of tissues, such as amoebocytes; or cells intermediate between these two types, as far as their independence is concerned, namely, ova and spermatozoa.

Part IV. The organismal differentials of tumors, which represent modified tissues.

Part V. The role played by organismal differentials in the maintenance of the harmony of the organism as a whole, and in the interaction of the organs and tissues within the organism.

Part VI. Immune processes in their bearing on the interpretation of organismal differentials. Organismal differentials as well as organ differentials may function as antigens and give rise to the formation of immune substances.

Part VII. The relationship between the evolution of species and organismal differentials.

Part VIII. The significance of individuality differentials in the psychical-social field; it is here that the concept of individuality had its origin.

in which there is only a relatively slight divergence in the constitution of the individuality differentials.

(c) *There are also differences in the reactions of different strains, belonging to the same species, against strange individuality differentials.* Such differences might be expected in the reactions between individuals from strains which have been inbréd to different degrees. The less close the inbreeding, the more severe will be the average reaction between different invididuals. Furthermore, a strain whose genetic constitution differs markedly from that of another strain may be expected to react strongly against individual members of the latter strain. But in addition, there is some evidence that different strains and also different individuals are able to react more strongly against strange strains and individuals than are others. Thus, among rats it seems that Busch strain rats reacted, on the average, more severely against individuals belonging to various strains than did strains of a different origin. Furthermore, among mice there is some indication that strain C57 tended to react more readily with lymphocytic infiltration of a strange tissue than did other about equally inbred strains. However, this observation is at present only a preliminary one; it needs further study and confirmation. There are also indications that certain individual animals exhibit a stronger reaction to the tissues of various other individuals than do other animals of the same strain. We must, therefore, in evaluating the significance of certain reactions as tests for the constitution of individuality differentials, consider the possibility that there exist some variations in the strength of the reactions which are independent of the degree of difference in the constitution of these differentials.

(d) *As to the differences in the reactions against different tissues,* all derived from the same individual and transplanted into the same host, these are quite marked. Tissues differ in respect to their resistance to injurious conditions and therefore in their ability to survive following transplantation. There are quantitative variations in this respect between different types of tissues. Some, as for instance, adult ganglia cells, which are severely injured by a short interruption of oxygen supply during and following the process of transplantation, cannot be successfully transplanted. Under ordinary conditions it is more difficult to graft, for any length of time, the adrenal cortex into a homoiogenous individual than the anterior hypophysis. Cartilage and perichondrium are very resistant to injuries associated with the process of transplantation; they withstand also relatively successfully an attempted invasion by lymphocytes and connective tissue, although even in this respect differences exist between very cellular cartilage and cartilage in which the intercellular substance predominates. Dense fibrous hyaline tissue resembles to some extent cartilage. Intermediate in their behavior following transplantation are kidney, fat tissue, salivary glands, and some glands with internal secretion, such as ovary and thyroid; and among each of these various organs different constituents are graded in their power of resistance, thus the excretory ducts are usually more resistant than the specific functioning parenchyma.

significance of the lymphocytes and polymorphonuclear leucocytes for the fate of the transplant; (4) the distant actions which, according to Blumenthal, the individuality differentials exert on the host after these differential substances have entered the host circulation. The connective-tissue and blood-vessel reactions occur at an early phase following transplantation and the character of these reactions is usually determined within the first two weeks. The lymphocytic reaction, as a rule, begins within the second week, but in some cases it may exert its full effect only at subsequent periods, and in certain instances, this reaction may appear and increase during the later phases of the interaction between host and transplant. The lymphocytes usually are indicative of finer differences between the individuality differentials; they are not found in any considerable numbers if there is complete compatibility between host and transplant, and they do not as a rule appear in very large masses if the incompatibility between host and graft is so great that the metabolism of the latter is seriously affected within seven to ten days following transplantation. But even in heterotransplantation these cells may accumulate after some time in the periphery of the injured graft. Polymorphonuclear leucocytes are seen in small numbers soon after the grafting of a piece of tissue, owing to circulatory disturbances and perhaps also to the presence of necrotic tissue, which is found under these conditions; but they accumulate in larger numbers usually only around and inside of heterogenous transplants. In the distant reactions, lymphocytes and polymorphonuclear leucocytes are activated in the circulation, under the same conditions under which they are activated locally around the transplant.

(b) *Differences in the mode of reaction against strange individuality differentials exhibited by different species.* While the factors which are involved in the struggle of an organism against strange individuality differentials are in principle the same in all the species with which we have worked, still, some quantitative variations exist in this respect. On the whole, rat and guinea pig react in a similar manner, although there may be minor differences in the intensity of the lymphocytic reaction in these species. There is, in addition, a stronger tendency on the part of the connective tissue to invade and replace transplanted fat tissue in the guinea pig than in the rat. There are, however, quite marked differences between the reactions in the guinea pig and rat, on the one hand, and in the mouse, on the other. In the mouse, the lymphocytic and connective-tissue reactions are in many cases less prominent and consequently the direct injurious action of the bodyfluids becomes more prominent. The amount of surviving tissue and the state of preservation of the transplanted cells are therefore largely indicative of the degree of compatibility or incompatibility of the individuality (organismal) differentials in this species. However, the lymphocytic and connective-tissue reactions may here also participate in determining the fate of the transplants and under certain conditions this participation in the struggle is quite pronounced and effective. In contrast to these species, in the chicken, in which the relative proportion of lymphocytes in the circulating blood is higher than in other species, the local lymphocytic reaction may be extremely strong even in cases

tion. In every case it is advisable first to study the sequence of events in the struggle of the host against the transplant, which sets in following transplantation and ends with the establishment of a new equilibrium of one or another kind. Thereafter, the time of examination should be, as far as possible, a constant factor in all the experiments. The time selected should be such that the effects of the injury, due to the process of transplantation, have disappeared, but the reactions have not yet progressed so far that finer gradations of the effects in different experiments have become impossible. The latter condition is very important, but it has not received due consideration by some investigators. As a rule, a period of 20 to 30 days following transplantation will be found most suitable for a comparison of the various tissue reactions and for the determination of relationship between the individuality differentials of host and graft. If the degree of incompatibility between host and transplant is only slight, a longer time may be required for the lymphocytic accumulations and infiltrations around the graft to become manifest, and in some cases collections of lymphocytes may appear even at a very long time following transplantation. However, in certain transplanted tissues the lymphocytic infiltration does not increase with increasing length of time after transplantation and there are some indications that in some instances it may even decrease in strength with advancing time. Whether this decrease in the effectiveness of the transplant with increasing time, which is especially noticeable after transplantation of cartilage, is due to a diminution in the amount of homoiotoxins produced or given off in the strange host, or whether it is due to an adaptation of the host to the action of the homoiotoxins, needs further study.

The choice of tissues to be used varies somewhat in different species. In guinea pig and rat, the simultaneous transplantation of thyroid with adhering parathyroid, of xiphoid cartilage together with the surrounding fat tissue, striated muscle, and bone and bone marrow, will make possible a satisfactory characterization of the relations of the individuality differentials of host and transplant. It may be of advantage to add a separate piece of striated muscle, thymus or salivary gland to the former tissues, all pieces to be implanted at the same time.

In the case of the mouse, the thyroid is not quite so useful a test tissue as in guinea pig and rat, because in the former species the reaction of the host against the transplant may in some instances consist merely in a shrinking of the graft, unaccompanied by the lymphocytic reaction which is so fine a reagent in the case of guinea pig and rat. But, also in the mouse a lymphocytic reaction may develop around grafts if incomplete compatibility exists and if the thyroid transplant remains, on the whole, well preserved. However, a shrinking of the thyroid transplant may take place also under other conditions, as when, for instance, the small thyroid of a very young mouse has been used for grafting, or when a part of this organ was injured during the process of transplantation. This multiplicity of factors, bringing about similar results, may make the analysis of the relation of individuality differentials in the mouse more difficult in some experiments. Therefore, in this species it is

In the ovary, the larger-sized follicles and corpora lutea are the most sensitive constituents and they are therefore the first ones to be destroyed after transplantation; in other cases, the small-sized follicles survive but do not develop to a larger size if the individuality differentials of host and transplant are not harmonious. Much more resistant than follicles is the germinal epithelium, which covers the ovary and usually forms a cyst after transplantation. Likewise, cortical spindle-cell connective tissue and medullary ducts, as well as germinal epithelial ducts, and also the epithelium and unstriated mucosa of the Fallopian tubes are more frequently able to withstand the injurious effects of homoiotoxin than even the small follicles. Most often the tissue remaining after destruction of all the others are strands of smaller and larger cuboidal cells, which are probably derived directly from the interstitial gland, and indirectly from the theca internal cells of atretic follicles; and these cells may be quite active as phagocytes and thus help in the removal of necrotic or hemorrhagic material. It may be remarked here that hemorrhages occur in certain transplanted tissues and also in some non-transplanted organs, such as the adrenal gland, probably more often than might be expected. The characteristics of the ovary in the mouse, which we have described, make this organ very suitable for the analysis of the individuality differentials.

Striated muscle tissue is fairly resistant and can be easily transplanted, whereas bone marrow is a rather sensitive organ that readily perishes. In contrast to ovarian tissue, testicle is sensitive. However, the power of resistance of analogous tissues may differ in different species; thus it seems that ovarian structures are more suitable for grafting in the rat and mouse than in the guinea pig. Different tissues differ also in their ability to grow after transplantation and also in their mode of regeneration, and these growth processes are inhibited by incompatibilities between the individuality differentials of host and transplant. Furthermore, there seem to be some differences in the quantity of individuality differential substances produced or given off by various tissues. Those possessing a very active metabolism, such as thyroid, produce these substances apparently in larger quantities than does cartilage. This conclusion is suggested by the fact that different tissues differ in the readiness with which they attract lymphocytes, and it may be assumed that the accumulation of lymphocytes is an indicator of the amount of active individuality differential substances.

2. *As to the methods which are most useful in the analysis of the individuality differentials,* the place of transplantation is important. It is necessary to select a place sufficiently large for the simultaneous insertion of multiple grafts, or, in other cases, for the serial transplantation of pieces of tissue, where, also, these operations can be dooe without serious interference with the health of the animals and where, moreover, the transplants can be recovered at the time of examination without much difficulty. Pockets in the subcutaneous tissue seem to be most suitable for this purpose, and by using this site in the majority of our experiments we avoided the introduction of unnecessary variations. In making the pocket it is important to avoid hemorrhages, which might interfere with the nourishment of the transplant in the period following opera-

effective in inciting a lymphocytic reaction than is cartilage with the surrounding fat tissue. Likewise, transplants of striated muscle tissue may be infiltrated with lymphocytes, when in other tissues lymphocytes are absent or much less numerous; but as a rule, also in the muscle tissue they are present in larger masses only if there is a definite antagonism between the individuality differentials of host and transplant. But lymphocytes accumulate very readily even around dead foreign bodies such as threads, especially if these foreign bodies are situated in tissues possessing an individuality differential which is not quite compatible with that of the host. In cases of infection in the fat tissue of the mouse, there may be found in addition to the accumulations of polymorphonuclear leucocytes, collections of lymphocytes, an increase in connective tissue and an infiltration of the fat tissue with small vacuolated epithelioid cells; but similar cellular changes may be noted in this animal also if the homoiogenous differentials of host and donor are sufficiently strange to each other. In such cases we have to deal either with a summation of the effects of incompatible individuality and organ differentials, or of the combined effects of the former and of bacterial infection or foreign body action. These complications by no means diminish to any considerable extent the value of the lymphocytic reaction as an indicator of the relation of the individuality differentials to each other even in the mouse, just as little as the value of the agglutination reaction in serological tests is destroyed by the fact that also changes in ion concentration in the medium in which cells or particles are suspended may cause agglutination; but it will be necessary to take all these factors into account in evaluating the results of such experiments.

In the large majority of our experiments we transplanted pieces of two or more different tissues from the same donor into different places of the subcutaneous tissue of the host and these pieces were subsequently removed at the same time for examination. As a rule, it was then found that the kind and intensity of the reaction of the host against these various tissues or organ pieces were similar. In autotransplantation, injurious reactions were lacking against all of them. In homoiotransplantation, if a severe reaction took place against one of the pieces, all the others were likewise severely damaged; if the homoiotoxins were less strong, the reactions in all pieces were equally mild. In syngenesiotransplantation, corresponding reactions of a mild character occurred in each case. In general, also the lymphocytic infiltration showed a similar degree of intensity in the different grafts from the same donor into the same host, provided the various complicating factors mentioned above were taken into consideration. Likewise, the reaction against all types of heterogenous tissues was of the same kind. In all these evaluations it is necessary to make allowance for differences in the sensitiveness, the power of resistance, the mode of growth of the tissues, and the amount of individuality differential substances produced in the various types of transplants. We should not expect the same reaction to take place against cartilage as against thyroid, as each of these tissues has its own peculiar characteristics. Because of the presence of so many variable factors present in investigations in which living tissues enter into various kinds of relations, it is necessary to make in each

advisable to transplant a larger number of tissues simultaneously, such as thyroid, xiphoid cartilage with associated tissues, ovary and striated muscle. A combination of this kind makes possible an accurate appraisal of the degree of compatibility in the large majority of cases. As a general rule, applying to all experiments of this kind, it is necessary to carry out the operations in a sterile manner, whenever this can be done, and to inflict as little injury as possible on the tissues.

4. *In the evaluation of the results of the experiments* various accidental complicating factors must be considered. Very important in this connection is infection with bacteria, which may occur notwithstanding the measures which may have been taken to avoid such an accident. In the majority of instances it is easy to recognize the effects of bacteria, with which the tissues were contaminated during the process of transplantation. The presence of localized masses of polymorphonuclear leucocytes around or in the graft indicates strongly the presence of extraneous mieroorganisms. However, in some experiments it may be difficult to decide whether the leucocytes may not have been attracted by sterile necrotic tissues. This difficulty is encountered especially in the mouse, where an infection may more readily take place, owing to the small size of these animals. Here we may find, in or around the transplants, either more scattered leucocytes or small accumulations of these cells, especially around the fat cells; these changes seem to be, on the average, more widespread and more intense if host and donor of the transplants are not nearly related, whereas, they usually remain localized when the degree of incompatibility between the individuality differentials of host and transplant is only slight; in cases of heterogenous transplantatioo, polymorphonuclear leucocytes are quite commonly attracted. There are certain other conditions when doubt may arise as to the significance of certain changes which have taken place in the transplant, as for instance, in case of injury of the transplanted tissues. Thus injury to fat tissue surrounding the xiphoid cartilage, caused by pressure of the forceps during the process of grafting, may lead to localized necrosis of fat tissue and cartilage; subsequently, the necrotic fat tissue may be replaced by fibrous tissue, and around the necrotic cartilage a plate of new perichondrial cartilage may form.

Another difficulty may be encountered when the thyroid of the guinea pig is transplanted. If this organ is surrounded by much fat tissue, the latter may prevent the ready ingrowth of capillaries from the host into the graft and the transplant may become necrotic over a smaller or wider area. Such a result is obtained especially if well-nourished, older animals, in which considerable amounts of fat tissue surround the thyroid, are used as donors and it will be necessary to guard against this complication. Further obstacles to a correct interpretation of the experiments may be due to the presence of adventitious factors, which may accelerate, intensify or retard the lymphocytic reaction. While the essential factor that determines the intensity of this reaction is the degree of incompatibility existing between the individuality differentials of bost and transplant, certain tissues are more prone to call forth a strong reaction than others. Thus in guinea pig and rat, thyroid tissue is usually more

if the results are somewhat less favorable, medium-sized or small follicles develop, and a still less favorable reaction is indicated if merely primordial follicles survive, without undergoing further growth processes; if the reactions are more severe, no follicles are seen in the transplant, but there may be only a cyst of the germinal epithelium, ducts, spindle-cell connective tissue and interstitial gland, together with necrotic remnants of the transplanted ovary; under the least favorable conditions, interstitial gland-like tissue may be all that is found, or even this may be missing and necrotic material and fibrous tissue alone may remain. However, it is always necessary to make allowances for the occurrence of accidental injuries to the transplants; but even if it should be difficult to recognize the latter, errors in the interpretation of the reactions can be avoided by performing a number of experiments, instead of relying on a single one. While this method of grading can claim only approximate exactness, still it is very helpful in comparing results obtained if different types of individuality differentials are made to interact.

In some cases we have used a second type of grades, which were as follows: Grade 6 is given to a typical autotransplant; grade 5 to a favorable, and grade 4 to a less favorable *syngenesiotransplant*; grade 3 to a milder and grade 2 to a severe homoiotransplant; grade 1 has the same meaning in both types of grades. These two systems of grading correspond to each other about as follows:

Type of Second Grades		*Main Grades*
6	=	3+
5	=	3 or 3−
4	=	2+ or 2
3	=	2 or 2−
2	=	1+
1	=	1

Unless specifically so stated, the first type of grading was used. In a general way, these series of reactions correspond to the spectrum of relationships extending from the autogenous through the syngenesious, first to the light and then to the severe homoiogenous reactions. Certain features indicating a still more severe injury are added in the case of heterogenous transplantations.

As to the terminology employed in distinguishing various types of transplantation, very frequently the terms: autoplastic, homoio- or isoplastic, and heteroplastic are used in the literature. However, the affix "plastic" accentuates the practical use which is made of transplantation in surgical "plastic" operations. But, transplantations may serve also as a method for the determination of the genetic relationship between the individuality differentials of host and donor, and then it would be more appropriate to designate these various types of transplantation as autogenous, syngenesious, homoiogenous and heterogenous, in order to emphasize the importance of genetic factors in such experiments.

Transplantations in which the relationships between host and transplant can

instance a large series of experiments in order to arrive at correct interpretations and to draw justified conclusions. Such experiments require, therefore, much patience and experience on the part of the investigator, but the problems are of sufficient importance to warrant these efforts. However, after all these conditions have approximately been satisfied, there remain still a number of variable factors in these experiments which have not yet been eliminated; therefore a complete solution of the problems involved is impossible at the present time and must be left to future work.

Since multiple transplants of various kinds from the same donor into a host behave in a corresponding manner, a grading of results can be made which express the degree of compatibility between the individuality differentials of donor and host, and thus a standard can be established with which to compare the results obtained in different experiments. The grades chosen for this purpose are arbitrary; they range between 1 and 3+ (3.25). In autotransplantation, the grades given are 3+ and 3. The tissues are well preserved and while at first there may be some irregularities in the structure of the grafts, they gradually assume more and more the characteristics of the normal organs, provided some accidental factors do not prevent such a development. Marked lymphocytic infiltration is lacking, but at early periods after transplantation some very small collections of lymphocytes may be seen; after some time, these cells usually disappear. Likewise, the connective tissue ingrowth into the autotransplant is restricted and an invasion of the fat tissue by small vacuolated cells and by fibrous tissue is lacking. Grades 3— (2.75) and 2+ (2.25) are given if the grafted tissues are, on the whole, well preserved, but if a reaction of the host is definitely noticeable, consisting in various degrees of lymphocytic infiltration and in a somewhat increased activity of the connective tissue, which may cause a rather mild injury to the transplant; reactions of this kind may be seen if donor and host are related to each other. If the reactions are somewhat more marked and tend to lead to a partial destruction of the transplant, grade 2 is given; this indicates a somewhat greater strangeness of the individuality differentials. In typical, more severe homoioreactions the grades range between 2— and 1. Grade 1 is applied in experiments in rats and guinea pigs, in which the thyroid has been entirely destroyed and the fat tissue largely replaced by fibrous tissue; grade 1+ signifies the survival of only a small part of the thyroid gland; the reaction in the fat tissue is still very severe. Grade 2— (1.75) is given if the thyroid gland is strongly invaded by fibrous tissue and a considerable part of it has been destroyed, but if at least one-half of the organ has escaped destruction at the time of examination. There is usually, in these cases, a definite lymphocytic infiltration, provided the injury to the tissues has not led to a marked diminution in the amount of the individuality differential substance present in or produced by the transplant. If in addition to thyroid, cartilage and fat tissue have been transplanted, corresponding grades may be given in accordance with the degree of survival of the tissues and the degree of lymphocytic infiltration. The addition of ovarian transplants may make possible a still finer grading: in the most favorable cases large follicles and even corpora lutea are found in such grafts;

if the results are somewhat less favorable, medium-sized or small follicles develop, and a still less favorable reaction is indicated if merely primordial follicles survive, without undergoing further growth processes; if the reactions are more severe, no follicles are seen in the transplant, but there may be only a cyst of the germinal epithelium, ducts, spindle-cell connective tissue and interstitial gland, together with necrotic remnants of the transplanted ovary; under the least favorable conditions, interstitial gland-like tissue may be all that is found, or even this may be missing and necrotic material and fibrous tissue alone may remain. However, it is always necessary to make allowances for the occurrence of accidental injuries to the transplants; but even if it should be difficult to recognize the latter, errors in the interpretation of the reactions can be avoided by performing a number of experiments, instead of relying on a single one. While this method of grading can claim only approximate exactness, still it is very helpful in comparing results obtained if different types of individuality differentials are made to interact.

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Transplantations in which the relationships between host and transplant can

be graded and in which a variety of tissues can be chosen for grafting provide the opportunity not only for the analysis of the organismal differentials of host and transplant, but also for the study of the interaction of various types of tissues and cells and of the factors which determine the tissue equilibria; they may therefore serve also as a method which may be of value in building up a physiology of tissues, in contrast to the physiology of organs which has been so extensively studied in the past. Accordingly, we shall emphasize this aspect of our investigations in describing our results.

Chapter 2

Autogenous and Homoiogenous Transplantations

We shall now compare the reactions against autogenous and homoiogenous transplants, first in rats and guinea pigs and then in mice and chickens, and we shall consider the transplantation of thyroid, cartilage and fat tissue, and later also that of striated muscle and a few other tissues.

1. *Autogenous transplantation of thyroid in rat and guinea pig.* After autotransplantation of thyroid in rat, as well as in guinea pig, there remains at first a ring of preserved acinar tissue around a central necrotic zone; the latter is smaller in the rat. Blood vessels and connective tissue cells are attracted by the transplant and the vessels and fibroblasts penetrate towards the necrotic center, where they form a loose, vascularized connective tissue. In the guinea pig, this takes the form of a distinct myxoid zone, situated between the necrotic center and the peripheral ring of living acini, whereas, in the rat this ringlike myxoid area is not so distinct. The organization of the center in the latter species is usually completed by the 15th day, when also the necrotic center of the parathyroid has been replaced by connective tissue; at this time, furthermore, accompanying the fibroblasts and vessels, a few lymphocytes or very small groups of lymphocytes may be seen in the central or peripheral connective tissue of the graft. In the first week after transplantation the colloid may be lacking in the acini of the transplant and phagocytes may contribute to the destruction of the latter, but after two weeks this colloid has been replaced by newly-formed colloid within the acini, the epithelium of which is rather low. The number of fibroblasts which move towards the center is, on the whole, rather small, and the few lymphocytes which may accompany these cells are probably attracted by non-specific factors, perhaps by the necrotic tissue; in other cases, foreign bodies may attract some lymphocytes. Around the 4th week, or somewhat earlier, the transplanted thyroid shows a curved structure. At this time an interesting change takes place, leading to the disappearance of the central connective issue which had been formed by the organization of the central necrotic material. This connective tissue, which may have become fibrous or hyaline, is either pushed out of the transplant into the neighboring tissue or it is invaded and absorbed by new fibroblasts of the host, although a small amount of hyaline material may remain within the inner ring of acini. The acini are surrounded by well-formed capillaries, not necessarily accompanied by fibroblasts. Towards the end of the 5th week, lymphocytes are either entirely lacking, or, in certain cases, are present in small collections. Between the 40th and 60th day the transplant begins to resemble the normal thyroid, but the epithelium is lower than in the original acini and a very small amount of fibrous tissue may be found between

some of them. In the lymph vessels, no lymphocytes, or only a few, may be seen. As in the rat, so too in the guinea pig the ingrowth of fibroblasts is limited and a loose connective tissue forms in the center adjoining the thyroid; but the latter formation, assuming the shape of a ring, is more pronounced here than in the rat. Also in the guinea pig the central fibrous tissue is eliminated after some time, and in both rat and guinea pig the blood vessels are attracted by autogenous substance; this conclusion is based on the fact that the vascularization is more marked in autogenous than in homoiogenous transplants; in addition, in the former, lymphocytes are lacking or slight in number. In rat as well as in guinea pig the injury to the acini and the destruction of colloid are gradually repaired and the transplants assume more and more the character of the normal gland. The activity of the connective tissue and lymphocytes is, therefore, restrained around and in autogenous thyroid grafts and this condition is best suited for the restoration of the normal tissue relations and of the normal structure of such transplants.

2. *Homoiogenous transplantation of the thyroid in rat and guinea pig.* The intensity of this reaction depends largely on the relationship between host and donor. In the rat, we carried out, therefore, three series of experiments, in which the average relationship was somewhat varied; the probability that a distant relationship existed between donor and host was greater in series B than in series A; in both of these series white rats were used. In series C, tissues of white rats were exchanged with those of cream and hooded rats. In all of these series the same factors co-operated in inflicting damage on the transplant, namely, (1) the action of the bodyfluids of the host, (2) the increased invasion by fibroblasts and lymphocytes and the increased production of fibrous or fibrous-hyaline tissue, which later interfered with the nutrition of the transplant and injured it by exerting pressure on it, and (3) the diminished supply of blood and lymph vessels, which also diminished the nourishment of the transplant. In series C the intensity of the reaction was greatest, and in series A it was slightly greater than in series B. In both the latter series the maximum of the reaction was obtained between the 20th and 30th day after transplantation, but in series B, where there was probably, on the average, a nearer relationship between the different rats than in series A, an improvement in the average condition of the transplants was observed from then on; this was lacking in series A and C.

In series A and B, at first conditions were similar. Between the 1st and 8th days, two or three layers of acini were preserved in a number of instances, but in others they were less well preserved; the acini were small and the colloid had been lost in many of them, while in others it was still present. Some capillaries grew through the ring of acini and mitoses were seen in the acinus cells. The necrotic center was organized by not very dense, small-celled connective tissue or by very dense fibrous tissue, or in certain cases some necrotic material was still left and was at least partly taken up by phagocytes. Hemorrhages from the rupture of the newly-formed capillaries may have occurred. From the 6th day on, lymphocytes appeared in series A, while in series B they accumulated somewhat later. They were carried to the transplant first by way

of the lymph vessels and they collected at the periphery of the center, or they filled the center diffusely; moreover, lymphocytes and also some connective tissue cells surrounded some acini. The central portion of the parathyroid was necrotic and in process of organization. Mitoses were seen in both thyroid and parathyroid.

In series A, between the 10th and 15th day, the thyroid transplant had disappeared, only fibrous tissue with some lymphocytes was observed in a number of cases. In other experiments there were some isolated acini or groups of acini embedded in masses of lymphocytes, which had accumulated and which gave to the transplant almost the appearance of a lymph gland. Lymphocytes could be seen penetrating between and into certain acini and destroying them. The center of the transplant was densely fibrous and blood vessels were here less conspicuous. As a rule, the thyroid ring, if present at all, was incomplete. After 16 and 17 days, variable amounts of thyroid tissue were found preserved; if the amount was small, the colloid was usually lost. Fibrous bands separated acini or bundles of acini and lymphocytes separated acini in certain areas; the center of the transplant and the lymph vessels were filled with lymphocytes. There was some indication also in this series that the homoioreaction was especially marked after transplantation in certain strains of rats.

Series B differed from series A in that, in the former, the reactions were on the average slightly less severe up to about 30 days after transplantation, but from then on, a diminution in the intensity of the lymphocytic reaction and in the destruction of thyroid issue set in, which was quite noticeable between the 40th and 85th days. While the lymphocytic reaction appeared somewhat later in series B, a marked lymphocytic infiltration did occur, and fibrous bands surrounded the acini during the earlier periods. In both series, in exceptional cases, the grafts showed the character of typical syngenesiotransplants, in which, instead of dense fibrous tissue, there was loose connective tissue with blood vessels in the center; here lymphocytes had accumulated as well as in the periphery of the graft and they penetrated also between the acini. But in other instances there was, in these cases, an intense destruction of the thyroid by lymphocytes and connective tissue.

In series C, a complete or almost complete destruction of thyroid and parathyroid had occurred at about 20 days after transplantation, the reaction being very severe, in accordance with the greater difference between the individuality differentials of host and donor. There are, then, indications that the intensity of the reactions against transplanted thyroid varies in accordance with the relationship between host and transplant.

In homoiogenous transplantations of the thyroid gland in the guinea pig, which were carried out with Hesselberg, the results were very similar to those obtained in rats. Yet some differences between autogenous and homoiogenous transplantations are brought out perhaps more clearly in the former than in the latter species. In a first period, lasting about four to five days and representing the earliest reaction to the injury, there is no marked difference between transplantation of autogenous and homoiogenous thyroid. During this interval the first mitoses appear and at the end of it some new acini may be produced.

There follows a second stage, one of transition, extending from the 5th to the 12th day, in which the formation of acini is less in homoiogenous than in autogenous tissue; likewise, colloid is newly produced in lesser amounts in homoiogenous than in autogenous grafts and this may perhaps be at least partly due to injury inflicted by the homoiotoxins rather than by the lymphocytes of the host. During this period, and still more so during the third period, beginning after 12 days, there is an increase in lymphocytes and connective tissue in the homoiotransplants; the fibroblasts tend to produce fibrous tissue, which separates groups of acini as well as isolated acini and exerts pressure on them; the lymphocytes accumulate in increasing numbers in the homoiotransplants and invade and destroy the acini. In the autotransplants, wider blood capillaries grow through the thyroid ring into the center and here the zone of myxoid connective tissue develops, which we have already mentioned, and it surrounds the central fibrous tissue, which is either converted into loose connective tissue by ingrowing capillaries and fibroblasts or is expelled into the surrounding tissue. At the same time, fat tissue may be pushed into the center of the thyroid from the outside and fat cells may be observed, although very rarely, also in the lumen of an acinus. This distribution of thyroid acini in the fat tissue is not found in homoiotransplants. At a later period, as, for instance, five months after transplantation, the autotransplanted thyroid may be almost like the normal, non-transplanted gland. Dense fibrous tissue separating the acini, as well as collections of lymphocytes, is lacking. The center of the graft consists merely of small amounts or strands of loose connective tissue. However, mitoses are not frequently seen in such transplants. The greatest number of mitoses is found from 7 to 9 days following autotransplantation and some mitoses may be found at this time even in homoiotransplants; they may still be frequent in autogenous transplants during the later days of the second week, but are lacking or very rare after 17 days.

In discussing the variable accidental factors which, apart from the nature of the individuality differentials, may influence the result of transplantation of various organs, we mentioned the fact that if together with the thyroid gland much fat tissue is transplanted, a great part or even the whole of the transplant may become necrotic. This seems to be true, however, only of homoiotransplants. The same factor interferes with autogenous transplants much less seriously; it appears that in the latter a partial necrosis caused by the fat tissue, may later disappear, as a further demonstration of the great power of adjustment possessed by autogenous tissue.

We find, in guinea pigs, that younger hosts react on the average in a milder way towards homoiogenous transplants than do older animals; thus in grafts made in hosts about 10 days old the preservation of the thyroid gland is, on the average, somewhat better and the formation of fibrous tissue in the transplant less extensive than in older hosts. Moreover, the lymphocytic reaction may be less severe; but if the preservation of the thyroid tissue is relatively good, the lymphocytic reaction may be quite intense in the young guinea pigs as well. Some differences between younger and older hosts may be noticeable between 11 and 17 days after transplantation. While as a rule also in younger

animals there are typical homoiogenous reactions, occasionally reactions appear which are more characteristic of syngenesiotransplants, and even as late as 20 to 25 days following transplantation the grade 3− was obtained in a younger host, which signifies that the structure of the graft approached that seen after autotransplantation. In older guinea pigs the reactions were more severe. However, we found that in different series of experiments with guinea pigs obtained from different breeders, the severity of the reactions differed somewhat, in the same way as in transplantations in the rat.

The grades obtained between 20 and 25 days after homoiotransplantation were 1, 1+, and 2−. Between 25 and 40 days, in the majority of cases, the grade was 1; in only one-third of the cases the grade 1+ (1.25), and in no case grade 2− (1.75), was reached. In younger guinea pigs the grades between 20 and 25 days were somewhat better; they varied between 1 and 2, and even grade 3− (2.75) was attained. Even after 50 and 60 days, while the grade was mostly 1, in a few cases grades 2−/1+ (1.50), or even 2/2− (1.87), were obtained.

3. *The effect of feeding thyroid substance to guinea pigs which are hosts of thyroid transplants.* If preparations of thyroid gland are added to the diet of normal guinea pigs, the mitotic activity of these transplants decreases and the colloid becomes hard, as an indication of the diminished function of this gland. However, when we gave, by mouth, daily 0.1 grain of thyroid to guinea pigs which received autotransplants of thyroid gland, the success of the transplantation was not diminished thereby, although the height of the acinar epithelium was low or medium to low. No increase in connective tissue nor in lymphocytes took place. Likewise, in guinea pigs with homoiotransplants of thyroid gland, thyroid feeding did not have any noticeable effect on the reaction of the host against the transplant. The grade in one guinea pig, after 30 days, was 1; after 20 days, the grades were: 1 in 6 guinea pigs; 2− in 2 guinea pigs; 2 in 3 guinea pigs, and in 2 additional guinea pigs it was 2 and 2− respectively. After 13 days, the grade was 1−. In a second experiment with younger guinea pigs the grades varied between 1 and 2. The effect which administration of thyroid hormone exerts on the thyroid gland did not, therefore, change the reaction of the host against transplants of this organ.

Autogenous and homoiogenous transplantation of cartilage and fat tissue in rat and guinea pig.

4. *Autotransplanted xiphoid cartilage in the rat,* as a rule, remains well preserved and the perichondrium is free of lymphocytes, in rat (as well as in guinea pig) only when the cartilage has been injured as the result of the operation, or is poorly nourished, owing to pressure or hemorrhage in the surrounding tissue or other injurious factors, it becomes necrotic. Thus, if the thick end of the cartilage near the bone is transplanted, the center of the transplanted piece may be at a disadvantage because of the lack of nourishment and may become necrotic and dissolved; subsequently, connective tissue may invade and replace the necrotic central areas. Necrotic pieces of cartilage may be surrounded by connective tissue which has grown into and replaced the transplanted fat tissue, or the perichondrial tissue may produce a new plate

of cartilage around the necrotic area, and in some instances the newly-formed cartilage may even infiltrate and take the place of the necrotic part. Correspondingly, central necrosis and solution processes may be found even in the normal, not transplanted xiphoid cartilage, in places where it is thick and where the central parts are therefore not well nourished. Transplanted fat tissue remains normal, but occasionally necrotic areas are found in it, presumably as the result of injury inflicted upon it during the transplantation; there may be at first very slight collections of lymphocytes, due probably to the presence of necrotic areas or to other accidental alterations of the fat tissue, but these disappear somewhat later.

5. *Homoiogenous transplantation in the rat of cartilage and fat tissue* produces the changes characteristic of homoiogenous individuality differentials. The same three series of transplantations were made in the rat with cartilage, bone and fat tissue as with thyroid. With thyroid, there was a grading of the intensity of the reaction, corresponding to the average degree of similarity or lack of similarity of the individuality differentials in these series. Upon this condition depended the amount of tissue destroyed and replaced by fibrous tissue and the intensity of the lymphocytic reaction. If we compare with these gradations in thyroid, the gradations in cartilage, bone and fat tissue transplants, we find in principle, in both kinds of grafts, the same condition, except that the differences in the power of resistance of different tissues caused some differences in the mode of reaction and in the absolute grades given. The greater portion of the cartilage could remain alive permanently in all three series of homoiotransplantations, but the fate of the fat tissue, epiphyseal cartilage, bone and bone marrow, and the frequency and character of the regenerative processes around necrotic parts of the cartilage served as indicators of the severity of the reaction and of the degree of compatibility of the individuality differentials of host and transplant.

Even in series C, in which host and donor of the transplant were least nearly related, the cartilage remained alive, and if there was localized necrosis a regeneration of perichondrial cartilage could take place, although this did perhaps not occur as regularly as in series A and B, and in some cases it led to the formation of a myxoid tissue instead of cartilage; this occurred if the intercellular cartilage substance was dissolved, while the cartilage cells remained alive. Evidently the development of toxic substances could inhibit the regeneration of cartilage. The epiphyseal cartilage and the transplanted bone were completely or almost completely necrotic in this series. However, there is some difficulty in the determination of the degree of necrosis of bone. If we adopt the condition of the bone cells as a criterion of the life or death of this tissue, then we encounter the difficulty that many of the apparently preserved and living bone cells may in reality be connective tissue cells, that had moved into the bone from the outside, in particular, from a zone of epithelioid cells surrounding the bone-cartilage border and perhaps representing merely fibroblasts, which, under the influence of bone tissue, assumed the character of epithelioid cells. But ordinary connective tissue cells may also invade and replace necrotic bone and cartilage. There was extensive necrosis in the trans-

planted fat tissue and replacement of the latter by fibrous tissue. Variable amounts of fat tissue could remain preserved, but it might be invaded by epithelioid and giant cells. Lymphocytic infiltration was usually marked in the fibrous tissue around the cartilage as well as around the blood vessels which supplied the fat tissue with blood, and also around the bone, but at other times the infiltration was moderate. In one transplant, even remnants of striated muscle tissue with nuclear chains were found. On the whole, the connective tissue and lymphocytic reaction against the transplant was considerable in series C, and more intense than in series A and B. Likewise, the bone marrow became more rapidly and more completely necrotic and it was more extensively replaced by fibrillar connective tissue than it was in the first two series. In series A and B, the necrosis of the fat tissue was less marked than in series C or it was lacking altogether, and there was often only localized ingrowth of connective tissue into the fat tissue, together with a moderate invasion by lymphocytes, which had a tendency to collect around the perichondrium.

As to the time relations in these reactions, they were about as follows: In the first three days after transplantation there was noticeable a movement of some polymorphonuclear leucocytes in the fat tissue, in the direction towards the cartilage; these cells disappeared in the following days. Between the 6th and 8th day, a new formation of cartilage could set in and a slight infiltration with lymphocytes, varying in strength in different specimens, took place. The reaction against the transplanted tissue usually was quite distinct on the 10th day after transplantation and the maximum could be reached between the 20th and 30th day. At this time, the average grade in series A was about 2— and in series B it was intermediate between 2— and 2. Between the 30th and 85th day a decrease in the average severity of the lymphocytic infiltration could occur, while the connective tissue reaction remained in a quiescent state. In some instances, at late stages the transplant even resembled an autotransplant, perhaps on account of an adaptation of the host to the originally strange tissue, a condition possibly akin to a state of active immunity.

In general there was, in the various experiments, a parallelism in the grades of thyroid and cartilage-fat transplants. In rats, in which the homoiogenous reaction was weak or lacking altogether in thyroid transplants, it was also as a rule lacking or weak in cartilage-fat transplants; but the destruction of the tissue by lymphocytes and connective tissue was, on the whole, much greater in the thyroid than in the cartilage; however, there were some instances in which the thyroid transplant was so markedly invaded by lymphocytes that it almost resembled a lymph gland, and then the cartilage-fat transplant likewise was severely infiltrated. On the whole, then, the principles which applied to the relationship between the individuality differentials of host and transplant and the reactions towards the transplanted tissues were about the same in the case of thyroid and cartilage-fat tissue.

6. *Autogenous and homoiogenous transplantations in the guinea pig,* of cartilage-fat tissue with the associated tissues, are very similar to the corresponding conditions in the rat. Again, in the first stages following transplantation there may be in both kinds of transplants, some polymorphonuclear leu-

cocytes, which later disappear. Fibroblasts and capillaries may grow into blood clots and organize them, and connective tissue cells may penetrate also into wounds or into necrotic areas in the cartilage. Some collections of lymphocytes around blood vessels or around the perichondrium and some increase in the connective tissue in the transplanted fat tissue in autogenous transplants may later disappear.

In the guinea pig as in the rat, homoiogenous cartilage may survive at least for as long as almost six months, and probably permanently, although slight degenerative changes or, in places, complete necrosis may take place in the intercellular cartilage substance in the course of the first or second week. During the second and third weeks, the lymphocytic infiltration may become quite marked, although this varies in different cases and even in different places in the same transplant. In some instances, towards the end of the third week, the lymphocytes may be so numerous that the cartilage becomes sequestered. During this time, also, the connective-tissue growth continues, leading either to a thickening of the septa in the fat tissue or to a substitution of fat tissue by fibrous tissue. In the second week, epithelioid and giant cells develop fairly often in the fat tissue and frequently perichondrial cartilage is formed around necrotic cartilage. It is of interest that in all the species examined so far, mitoses are rarely found in perichondrial tissue and in young cartilage cells, and only once was a mitosis seen in a perichondrial cell in the guinea pig. During the fourth week the homoiogenous reaction is fully developed. Not only may newly-formed cartilage surround the necrotic area, but also connective tissue with lymphocytes, with or without blood capillaries, or lymphocytes without connective tissue may penetrate a necrotic area in the cartilage and replace it. Lymphocytes may infiltrate and destroy part of the perichondrium, but they penetrate the hyaline intercellular cartilage substance not at all, or merely for a short distance. They do not destroy healthy cartilage to any large extent. On the other hand, cellular cartilage may more readily be invaded by lymphocytes and, at least in part, be destroyed. But on the whole the lymphocytic infiltration in cartilage-fat transplants is moderate and it is found especially around the living cartilage, in places where dense fibrous tissue surrounds cartilage or perichondrium; but there may be much lymphocytic infiltration also in preserved fat and areolar tissue. During the second month conditions are similar and lymphocytes may now also move lengthwise in the direction of the fibrillation in the cartilaginous ground substance and here they gradually perish. The injurious action of the lymphocytes makes it occasionally possible for the blood vessels and connective tissue cells to penetrate for a short distance the marginal portion of the cartilage. Otherwise, connective tissue cells push only into necrotic cartilage. After 5½ months, the reaction on the part of the host cells was not more intense than at earlier periods and the lymphocytic reaction over wide areas could be mild; likewise, some fat and areolar tissue could still be preserved. The homoiogenous bone marrow had become necrotic and was replaced by a loose fibrillar connective tissue; bone was surrounded in places by giant cells.

In general, in homoiogenous thyroid and fat tissue the activities of the con-

nective tissue and lymphocytes set in at about the same time, and in the same host the relative degree of the lymphocytic reaction was in many cases the same in various tissues, despite the fact that cartilage and fat tissue give off a smaller amount of homoiogenous substance than do thyroid and kidney. Although it seems that lymph vessels grow more actively into the thyroid than into fat tissue, it is not probable that this explains the difference in the degree of lymphocytic reaction in these two tissues, especially in view of the observation that the lymph vessels which grow into the homoiogenous fat tissue are less crowded with lymphocytes than are those in the thyroid gland.

Autogenous and homoiogenous transplants of cartilage-fat tissue differ, then, not only in the greater ability of the former to survive and the great injury inflicted on the latter by the homoiotoxins, as well as by various kinds of host cells, but also in the greater regulative power in the autogenous tissues which successfully overcomes the results of injuries caused by the experimental procedures used. However, also homoiogenous cartilage, perichondrium and fat tissue possess to some extent still a certain regulative power, as indicated by the new formation of perichondrial cartilage around an area of necrotic cartilage. In autogenous and homoiogenous transplantations conditions are therefore very similar in rat and guinea pig. A first period of injury and degeneration is followed by a second period of recovery and regeneration, which affects the same tissues. There are, however, some minor differences, mainly of a quantitative nature, in these two species. In the rat the regenerative activity of the perichondrial cells seems to be greater than in the guinea pig; but in the latter the invasion of fat tissue by connective tissue, as a rule, is more extensive than in the rat. On the other hand, in the rat the lymphocytic reaction may be somewhat more intense.

We have, so far, discussed autogenous and homoiogenous transplantations of thyroid, cartilage and fat tissue and associated tissues in rat and guinea pig; these were the tissues commonly used in our investigations. But in addition we have made use of a number of other organs or tissues; from among these we shall select striated muscle in the rat, and uterus and kidney in the guinea pig, for a comparison of autogenous and homoiogenous reactions. Each of these organs shows some peculiarities which are of interest in the analysis of the common factors underlying the differences in the reactions against autogenous and homoiogenous individuality differentials.

7. *Autogenous and homoiogenous transplantation of striated muscle tissue in the rat.* Elson found that after autogenous transplantation of striated muscle tissue, the latter remains preserved for at least six months, and probably indefinitely. During the first few days the greater portion of this tissue became necrotic and was invaded by polymorphonuclear leucocytes, which were attracted either by the necrotic material or by some accidental bacterial products; they disappeared again after a few days. After four days a proliferation of the muscle nuclei set in, and this was quite marked after six days, the nuclei lying in long slender muscle spindles, many of which developed cross-striations. Gradually the latter became more definite, while some of the nuclei disappeared and others assumed a more peripheral situation. At fifteen

days, the muscle tissue consisted chiefly of long slender fibers, with good cross-striations and an increase of muscle nuclei as compared to the normal number. By this time the lymphocytes, which at first were present in small numbers, had almost or completely disappeared. At 28 days the muscle, except for slight signs of degeneration in a few areas, appeared mature and normal in almost every respect. Conditions were similar when the transplanted muscle was about at the height of its development. Later on there was some invasion by fibroblasts and the muscle fibers became small; but there was still some increase in nuclei and in connective tissue cells and lymphocytes. It was presumably the abnormal situation and the lack of the normal function of the transplanted muscle which led to the slight pathological changes noticeable at this time.

After homoiotransplantation of striated muscle tissue there were fewer regenerative growth processes in the first period and much more degeneration, on account of an invasion of the transplants by lymphocytes, and, to a less extent, by connective tissue at subsequent periods. Thus homoiotransplanted muscle disappeared much earlier than autotransplanted muscle, no well-preserved tissue being present at 50 days. This result was due to the primary action of the bodyfluids of the host, which were inadequate for the homoiogenous graft, which injured it and interfered with its growth processes; and it was secondarily the result of the activity of the host cells, which led to further and, in the end, total destruction of the muscle tissue, at a time when the autogenous muscle was well preserved. The growth processes, which take place in the muscle following a primary degeneration of its major portion, represent less true regenerative processes than those of compensatory hypertrophy, consisting in an increase in sarcoplasm and a multiplication of nuclei.

While in the autotransplanted muscle a nuclear proliferation was seen as early as four days after transplantation, it was lacking at this time in the homoiotransplanted muscle. In the latter there appeared at six days a slight lymphocytic reaction and there were also fewer muscle fibers and a smaller number of well developed nuclei in the homoiotransplants. At ten days the lymphocytic reaction increased in intensity, some muscle fibers degenerated, but other muscle fibers remained and underwent still a slight proliferation of nuclei. There was thus, in the early periods after transplantation, a balancing between growth processes and degenerative processes. Later, the invasion of the muscle by lymphocytes and its destruction increased. At 32 days there was an intense lymphocytic reaction, which more or less completely took the place of the muscle transplant. However, there were a few small nucleated muscle spindles or fibers with cross-striation. The invasion of lymphocytes continued to increase and at the same time connective tissue cells of the host participated in the process of destruction of the graft. At 50 days, only a few remnants of muscle tissue were found, and at 70 days there was a maximum of lymphocytic reaction coinciding with a minimum in the preservation of the muscle tissue. At 118 days, no muscle tissue was seen; its place had been taken by lymphocytes, connective tissue and a few small blood vessels.

Hence, while the type of growth processes that occur in the muscle grafts is

the same after homoio- and after autotransplantation, these growth processes were less intense after homoiotransplantation and, instead, degenerative processes predominated, largely due to the activity of lymphocytes and connective tissue. But there was here also a primary injury of the transplant by homoiotoxins carried to the graft by the bodyfluids, which corresponds to the finding of Hesselberg in the transplanted thyroid of the guinea pig, and which has been observed by us also in this species in transplanted unstriated muscle tissue of the uterus and in the placentoma formation in transplanted pieces of uterus. The peculiarity of the growth processes in muscle grafts, which tend to repair the degenerative processes, consists in the fact that they have to contend with factors unfavorable to growth, similar to those which are found in liver tissue or in those parts of the epidermis which are farther removed from the source of nourishment, or in the connective tissue in the neighborhood of foreign bodies.

8. In experiments carried out with Hesselberg and Kerwin, *autogenous and homoiogenous transplantations of the uterus were compared.* Pieces of uterus were transplanted either into subcutaneous pockets in the abdominal wall or in the ear of the guinea pig. The latest period at which homoiogenous pieces were found in pockets in the ear was after 16 days, and in the abdominal wall, after 24 days. In the former, conditions for the survival of the transplant are more unfavorable than in the latter; at later periods, only hyaline tissue with some clusters of lymphocytes were found as remnants of the homoiotransplants. The autotransplants were well preserved after 35 days and would presumably have lived indefinitely. At that time they showed good preservation of the uterine surface epithelium and the glands, in both of which there was mitotic proliferation; strands of connective tissue separated groups of glands, without compressing them, because the connective tissue remained cellular-fibrillar or myxoid near the epithelium, without becoming fibrous; also, the unstriated muscle tissue was well preserved and in connective tissue as well as in unstriated muscle occasional mitoses were found.

Three periods can be distinguished as far as the fate of these transplants is concerned. In the first five or six days there is no marked difference between autogenous and homoiogenous transplants. During the second period, lasting from the 6th to the 20th day, differences develop between the autogenous and homoiogenous tissues, and in the third period the latter are in the process of destruction, while the former are well preserved. In both auto- and homiotransplants the tissue is shrunken during the first few days, owing to the insufficient nourishment provided during this period; they not only recover from this condition later, but a new formation of tissue, as indicated by the occurrence of mitoses in various tissues, takes place. Also, the connective tissue recovers; it has a myxoid, cellular character near the epithelium and it shows mitoses. A part of the connective tissue is derived from the transplant, but other growing connective tissue has its origin in the host. The second period begins at about the 6th day, and on the 7th day, when the transplanted uterine epithelium forms a cyst with papillae, differences between the autogenous and homoiogenous transplants set in. Mitoses are found in the cellular-myxoid

connective tissue in the autotransplant and, in general, there are many mitoses here. In the homoiotransplant the epithelium is flatter, there are more lymphocytes and the connective tissue has a more fibrillar-cellular character. The unstriated muscle tissue is either lacking or it is invaded by connective tissue in the homoiotransplant. However, certain variations as to the differences between autogenous and homoiogenous transplants occur in individual cases; but on the whole, the recovery of the autogenous tissue is better and especially the myxoid connective tissue and unstriated muscle are better preserved in the autotransplant and there are here fewer lymphocytes. In the homoiotransplant, the epithelial cyst remains incomplete and there is more necrotic tissue, but even in this kind of graft the lymphocytes are not present in large masses and they are not very injurious during the second week and first half of the third week, but they may destroy the uterine glands and injure the surface epithelium.

In the homoiotransplant the connective tissue still invades the unstriated muscle tissue and gradually during the latter part of the third and the beginning of the fourth week the lymphocytes become more frequent around and in the graft, and very little myxoid connective tissue or muscle tissue is, at this time, to be observed. The surface epithelium, some glands, and the peritoneal epithelium are more resistant to the action of the homoiotoxins than are the myxoid connective tissue and the unstriated muscle tissue. On the other hand, the amount of hyaline connective tissue increases, at least partly on account of the progressive organization of the necrotic material. In the middle of the 4th week, the latest period at which living homoiotransplanted tissue was seen, few mitoses were found in the epithelium, although this epithelium and the peritoneal endothelium were relatively best preserved, while the myxoid connective tissue and the muscle tissue were replaced by fibrillar and hyaline connective tissue.

It follows from these experiments that great parts of the homoiotransplanted uterus are primarily damaged by the action of the homoiotoxins, that uterine tissues attract the lymphocytes less strongly than do kidney and thyroid, and that these cells are of less significance in uterus than in thyroid and kidney. In uterus transplants the lymphocytes contribute only secondarily to the destruction of the graft and then chiefly through their action on the epithelial structures. Thyroid and kidney are largely epithelial structures, they are less affected by the bodyfluids of the homoiogenous host, although a primary effect of the strange bodyfluids may be exerted also on these organs; but, in the main, their destruction is brought about by lymphocytes and connective tissue cells. It is probable that without the injurious action of these cells of the host, the homoiogenous thyroid and kidney would survive longer than the homoiogenous uterus transplants. The latter tissues cannot recover as well from the primary injury caused by the process of transplantation, nor can their epithelium induce or maintain the myxoid-cellular character of the stroma as well as autogenous tissues. Furthermore, the tissues that have recovered cannot maintain themselves permanently in the homoiotransplants because of the inadequacy of the bodyfluids. The changes produced in the stroma react un-

favorably also on the epithelium and a vicious circle is thus established in the epithelium-stroma relations. Also, the uterine epithelium is not seriously affected by the homoiotoxins, if at all; but secondarily, the epithelium is injured by the lymphocytes. While the lymphocytes appear at about as early a time in or around the homoiogenous uterine tissues as around the corresponding thyroid and kidney tissues, in the latter the lymphocytic reaction becomes much stronger than in the former. The resistance of the epithelial structures in the homoiogenous uterus is indicated also by the fact that mitotic proliferation continues actively in the transplanted uterine epithelium much longer than in thyroid and kidney.

9. *The effect of autogenous and homoiogenous bodyfluids on the development of placentomata in the transplanted uterus.* If the non-transplanted uterus has been sensitized by the corpus luteum hormone about five to eight days after estrus, incisions into the uterine horn or introduction of foreign bodies into the uterus call forth the production of placentomata, which reach their full development in about ten days, while at a still later period regression of these newformations sets in. If instead of making incisions into the uterine mucosa in situ, we autotransplant pieces of the sensitized uterus about six days after estrus, the mechanical stimulation due to the process of transplantation likewise leads to placentoma formation in the transplanted periglandular connective tissue, which proliferates actively by means of mitoses. At the height of the development, pearls and also giant nuclei may form in certain areas in the placentoma; the other uterine tissues are well preserved and lymphocytes are absent, but some spindle-cell connective tissue with mitoses may grow into the placentoma and destroy parts of it.

If pieces of the uterus are homoiotransplanted instead of autotransplanted, the results differ in accordance with the availability of corpus luteum hormones in the host; however, even under the most favorable hormonal conditions the homoiotoxins always tend to exert an injurious effect on the transplants. If the homoiotransplantation is made into guinea pigs, in which estrus has taken place about six days previous to transplantation, in the large majority of the animals only traces of or very slight placentomata developed, or none. Occasionally, large placentomata developed, but in this case there seemed to be more necrosis in these homoiogenous than in the autogenous structures; also, moderate or even marked lymphocytic infiltration could be found about 10 or 11 days after transplantation in some of the connective tissue of the transplant.

In five pregnant guinea pigs, pieces of uterus were homoiotransplanted. In two animals no placentomata developed, while in three there were small placentomatous areas. Lymphocytic infiltration in the surrounding tissue was moderate, or, in places, more marked, and it was found also directly in the placentomatous formations; again, the ingrowth of spindle-cell connective tissue could destroy and replace part of the placentomata. After transplantation of the uterus pieces into male or into sexually immature female guinea pigs, no placentomata developed and the degree of lymphocytic infiltration varied in different cases.

In the development of placentomata in the homoiogenous uterus of the guinea pig we see another example of a direct injurious action of the homoiotoxins present in the circulating bodyfluids, which damaged the mucosa of the uterine transplant to such an extent that the formation of placentomata was either prevented or much reduced under conditions in which normally the sensitizing hormones would have made these growth processes possible. There was, then, a struggle between the sensitizing hormones and the homoiotoxins; only in a few instances was the hormone action, in conjunction with the mechanical stimulation due to the trauma, able to overcome the injurious effect of the homoiotoxins. The lymphocytic infiltration was quantitatively so weak that the reaction on the part of the host cells could not be held responsible for these growth-depressing effects.

10. *Transplantation of autogenous and homoiogenous kidney tissue.* The process of transplantation initiates tissue reactions in the host and changes in the transplant. These may be due to general factors common to autogenous and homoiogenous tissue; in addition, there are the specific reactions due to the varying degrees of incompatibility of the individuality differentials of host and transplant. These differences in individuality differentials activate the formerly quiescent host tissues in accordance with the inherited, constitutional characteristics of the latter. Furthermore, by comparing the reactions in tissues transplanted into different locations, the general injury to the tissues is found to vary in degree; in a particularly unfavorable situation, with increasing injury to the transplant, the general, less specific reactions of various tissues which are caused by the injury, may dominate over the more specific reactions which are induced by incompatibility between the individuality differentials of host and transplant. It is in order to analyze still further these characteristics of tissues that we shall record the principal observations as to the differences between autogenous and homoiogenous transplants of kidney tissue in the guinea pig. Greater damage is suffered by these grafts, for instance, after transfer into pockets in the ear of guinea pig than into pockets in the subcutaneous tissues of the abdominal wall or of the dorsum. This is true of both autogenous and homoiogenous tissue and both may die after some time, although the latter is earlier destroyed; also, the specific differences between these two types of transplantation are less evident in ear transplants than in those in the subcutaneous tissue of the anterior or posterior wall of the abdomen. Likewise, homoiotransplants of uterine tissue are destroyed more rapidly in the ear than in the abdominal wall.

After transplantation into the ear of pieces of kidney, destruction of homoiogenous tissue was complete after 21 days, while the autogenous tissue, although injured by the ingrowing connective tissue, was not yet quite destroyed at 38 days. Transplanted into the subcutaneous tissue of the abdominal wall, both autogenous and homoiogenous kidney tissue was preserved for at least 30 days, and it probably remained alive for a still longer period. These differences were presumably due to the greater pressure exerted on the grafted tissue in the ear and, perhaps, also to a less satisfactory blood vessel supply in this region. The same factors caused also a much more marked lymphocytic

infiltration of the homoiogenous tissue in the back of the abdominal wall than in the ear. In the autogenous transplants there were occasional lymphocytes around the tubules or in the capillaries of glomeruli, especially in places where the connective tissue was increased around the tubules, but often they were entirely absent, while they were very prominent in homoiogenous pieces, especially in those transplanted into the abdominal wall.

The lymphocytic infiltration is distinct after nine days in homoiotransplants, after which period it increases gradually—this applies also to transplants in the ear, although here it is less prominent. Lymphocytes collect around the kidney transplant and penetrate into the lumen of the tubules as well as into the glomeruli, isolating and helping to destroy and replace the tubules. The destruction of tubules by these cells can be seen at ten days after transplantation and it persists from then on. In the central necrotic or organized area they are present in smaller numbers. However, the intensity of the lymphocytic reaction varies in different homoiotransplants; it apparently is not prevented nor even noticeably diminished by a loss of even as much as one-half of the weight of the host. This reaction was similar in strength in thyroid and in kidney; in both these organs, after homoiotransplantation, it became noticeable at about the same time and gradually progressed. If a bacterial infection takes place, the tissue at some distance from the place of infection may remain preserved, and the infection does not call forth a lymphocytic reaction in autogenous transplants nor does it noticeably increase it in the case of homoiotransplants; but ordinarily already the lymphocytic reaction is marked around and in homoiotransplanted kidney. As we have stated, in homoiotransplanted tissue of the mouse it seems that bacterial infection may increase the intensity of the lymphocytic reaction.

While the degree of compatibility between the individuality differentials of host and transplant largely determines the reaction on the part of the lymphocytes, the less specific factors of injury sustained by the transplants during and following the transplantation, the unfavorable conditions in the transplant, caused by its strange situation, and the difficulty of entering into normal relations with the new tissues surrounding it are, to a greater extent, responsible for the activity of the fibroblasts and the production of fibrous tissue. The organismal differentials are of less significance in these latter changes, although they are still important. In both autogenous and homoiogenous tissues the connective tissue grows between the tubules and glomeruli of the peripheral zone and progresses towards the central necrotic material. However, a larger number of fibroblasts seem to grow into the homoiotransplants; this is especially clear following the first two weeks, when the injury due to the operation has in great part subsided. In the autogenous transplants the closely approximated tubules are arranged in lobules and it is the lobules which are separated by connective tissue, whereas, in the homoiotransplants the individual tubules are separated by connective tissue; but strands of connective tissue may surround some individual tubules also in autotransplants. Through the exertion of pressure by this tissue the lumen of the central tubules is obliterated or shrunken. But in addition there may be atrophy as a result of insuffi-

cient nourishment. The mechanism underlying these relations of the connective tissue to various types or degrees of injury of the parenchyma is, as yet, very little understood. Stereotropic reactions, movements in contact with strange foreign bodies, perhaps chemotropic activity elicited by necrotic tissue, as well as other altered relations between the more specific parenchymatous tissue and the surrounding stroma, may form the stimulus for both movements and increase in size and multiplication of cells in the surrounding connective tissue. It is these factors which may ultimately lead also to the destruction of autogenous skin cysts in the guinea pig, which form after subcutaneous transplantation of epidermis with the underlying connective tissue. Here the surrounding connective tissue may grow stereotropically along the hairs of the skin into the interior of the cyst, where it separates and surrounds the keratin lamellae. Thus after some time the host connective tissue may bring about the destruction of autogenous subcutaneous skin transplants; but in addition there develop specific lymphocytic reactions around homoiogenous skin grafts.

The size and character of the central necrotic area in transplanted kidney varies in different animals; the organizing fibroblasts grow first between and along the necrotic tubules and then into the tubules. As the result of the organization of the fibroblasts the necrotic material becomes hyaline; in addiion, the latter is taken up by phagocytes, which thus aid in its removal. On the whole, on account of the relative hardness of the necrotic kidney tissue, the organization progresses slowly and with unequal rapidity in different hosts; it may require 20 days or even longer for this process to be completed. Following organization of the autotransplanted thyroid, as we have stated, the central necrotic part after some time becomes cellular and small in size, but the homoiotransplanted thyroid is fibrous-hyaline and large. However, in the kidney there is no marked difference between the condition of the central parts in autogenous and homoiogenous transplants, because in both, the necrotic kidney tissue is resistant to the action of the organizing host cells. The principal difference between the two types of kidney transplants consists in the number of lymphocytes and of fibroblasts and in the amount of connective tissue in the peripheral living area.

The tubules in the transplanted kidney are simple and similar to the collecting tubules. The glomeruli are small and tend to be hyaline. There are frequent mitoses in the tubules between five and seven days; at nine days a mitosis was found in a glomerulus. From the 7th or 8th day on, a decrease in their number occurs, but they are found even after 27 days, although not after 30 days, in both autogenous and homoiogenous transplants. The mitotic activity may, at least for some time, be as great in the latter as in the former, or even greater. As a result of the transplantation a chain of changes is initiated in the transplant, irrespective of the character of the individuality differentials, and these changes lead to mitotic cell proliferation; gradually this reaction curve declines, and at last a new equilibrium is reached in which cell proliferation ceases, although the original normal condition of the transplant has not been restored. These curves of changes are similar in thyroid, kidney and uterus transplants, although some differences exist. It is possible that in homoio-

transplants, to the primary injury which initiates the regenerative processes is added the secondary injury inflicted on the tubules by lymphocytes and connective tissue cells; this second factor may cause increased mitotic proliferation, provided enough tubule tissue is left to respond with such activity.

We may then conclude that the difference between autogenous and homoiogenous transplants of kidney pieces in the later stages is due to the more rapid destruction of the regenerating tubules in the latter grafts, rather than to a primary difference in the actual development and growth of kidney tissue, which almost ceases on or about the 14th day after transplantation, although a slight regenerative process may occur at a still later date. The damage to the tissue begins on about the 9th day and in the homoiotransplant destruction is almost complete 21 days after transplantation. The activity of the connective tissue destroys also the autotransplant, which has been injured through the abnormal conditions in which it lives, especially in the ear; this activity is therefore, at least partly, an injury reaction, which may develop in autogenous as well as in homoiogenous transplants. In contrast to the connective tissue cells, the lymphocytes react more specifically to homoiotoxins which are present in the bodyfluids of the host and in the transplanted tissue itself; these toxic substances are both involved, partly directly, partly indirectly, in the activity of the lymphocytes, but, to a certain extent, also in that of the connective tissue elements.

Chapter 3

Transplantation of Autogenous and Homoiogenous Tissues in Mice

THE LARGE majority of experiments which we carried out in mice were done with closely inbred strains, and exchange of tissues between members of the same strain would therefore not correspond to homoiogenous transplantations but to something akin to autogenous or syngenesiotransplantation. Which of these two types of transplantation it resembles more would depend on the degree and effects of the inbreeding. However, experiments in which tissues are transferred from one strain to another strain would be more nearly comparable to homoiogenous transplantations, although there is no absolute identity of inter-strain transplantation and ordinary homoiotransplantation; in the latter there may be a somewhat greater variability in the relations between the individuality differentials of host and transplant. In addition to the transplantation between different strains—inter-strain transplantation—we have also made some experiments in which tissues were exchanged between ordinary non-inbred white mice obtained from various dealers. As to autogenous transplantations, these should not be affected by the inbreeding and should yield the same results in closely inbred, in less closely inbred, and in non-inbred strains.

Autogenous transplantation in mice. Autogenous transplantation is in all essential respects similar to this type of transplantation in rat and guinea pig. The tissue remains preserved provided the injury connected with the process of grafting and that due to the abnormal position of the graft have no long-lasting, unfavorable effects. The changes which are observed after autotransplantation can not be due to incompatibility between the individuality differentials of host and transplant, since these differentials are identical, but they are due to mechanical or chemical factors of a non-specific kind, similar to those which, under corresponding conditions, might also take place in non-transplanted tissues. On account of vascular changes around the transplant and of necrosis in the insufficiently nourished portion of the grafted tissues, polymorphonuclear leucocytes may appear; lymphocytes may be attracted by non-specific factors, such as foreign bodies, causing a mild degree of injury, and epithelioid and giant cells may be produced. Injury or abnormal growth processes in non-transplanted normal striated muscle tissue may call forth a multiplication of muscle nuclei and the formation of thinner muscle fibers or spindles. The same changes may take place in transplanted muscle. Dense fibrous tissue tends to form around and sometimes between the living muscle fibers, and, at first, some lymphocytes may accumulate between the muscle fibers. But the lymphocytes were not numerous in the autotransplanted muscle tissue; they were still found at 20 days, but no longer after 30 days following

transplantation. Hemorrhages were observed in the thyroid transplant at 12 days, therefore at early periods after the operation, but they also had disappeared at 30 days. In one autotransplant of thyroid gland an abscess, due to bacterial infection, adjoined the graft in one place; the ring of acini was interrupted at this point, but this condition did not destroy the autogenous character of the transplant except locally. In early periods following autotransplantation the amount of fibrous tissue in the center of the graft may be considerable, owing to the organization of the central necrotic tissue; but this decreases later. In the transplanted fat tissue, infiltration with lymphocytes, a noticeable increase in connective tissue, and the presence of small vacuolated phagocytic cells are lacking. Localized necrosis of the cartilage may here also be followed by the formation of a plate of new cartilage through the regenerative activity of the perichondrium. We have not examined autotransplanted mouse tissue later than 30 days following transplantation, but there were already some indications at this period that the effects of the accidental factors we have mentioned disappear in the course of time as a result of regulatory activities of the tissues, which take place under autogenous conditions. By a comparison of the results of autogenous and homoiogenous transplantation it is thus possible to separate a variety of more or less accidental factors from the specific ones caused by the disharmony of individuality differentials.

Homoiogenous transplantation. This consisted of two kinds of experiments, namely, (1) an exchange of tissues between not closely related tame mice or between non-inbred and inbred strains of mice, and (2) exchange of tissues between closely inbred strains of mice. The second set of experiments was first carried out, and in this type of homoiogenous transplantations the connective-tissue as well as the lymphocytic reaction was definitely weaker than in the corresponding transplantations in rats and guinea pigs. There was the possibility that the relatively low intensity of these reactions was due to the close inbreeding to which these mice had been subjected. We added, therefore, to these transplantations, the first series; but here the results were similar, indicating that these weak reactions are characteristic of the mouse and that they are not due to the close inbreeding. If we make allowance for these differences, the grades in these transplantations in mice are otherwise in principle the same as in the experiments with rats or guinea pigs. If the thyroid was preserved, the relative incompatibility of the individuality differentials in host and graft was indicated in many cases by the stunted condition of this transplanted organ; in addition, the organization of the central necrotic material was, in some instances, as yet imperfect. There were a number of experiments in which, particularly in the fat tissue, there were found either some scattered polymorphonuclear leucocytes or even small collections of these cells. In such transplants there were, as a rule, also an increased amount of fibrous tissue and an increase in lymphocytes visible in addition to small vacuolated cells. More rarely, a few polymorphonuclear leucocytes were found also in homoiogenous thyroid or other homoiogenous transplants. As already mentioned, there was often some doubt as to whether the presence of the leucocytes was not due to accidental infections with bacteria, which could take place more

readily in mice than in rats or guinea pigs, because of the greater difficulty of performing a perfectly sterile operation in the smaller animal.

In transplantations of pieces of thyroid, striated muscle, xiphoid cartilage with fat tissue, and of ovaries between not closely inbred mice, obtained from different dealers, or from inbred D or C57 mice to the former, after 20 and 30 days the grades ranged between 1 and 2—, except in one transplantation, in which the grade was 2. With the exception of the latter, the results were therefore characterisitc of severe homoio-reactions. In no case was ovarian tissue preserved. When grade 1 was given, neither the muscle nor the thyroid transplant was preserved; only cartilage and perichondrium had survived and the fat tissue was partly infiltrated with small vacuolated or epithelioid phagocytic cells and with varying amounts of fibrous tissue and lymphocytes; however, these reactions in the fat tissue were always much diminished as compared with those in rats and guinea pigs, except in some transplants in which polymorphonuclear leucocytes were more pronounced. In general, the amount of fat tissue preserved was greater in the mouse than in the two other species. In the case in which grade 2 was given, the thyroid transplant, while small, was in a relatively good condition; the center was filled with dense hyaline tissue and the surrounding ring of acini was incomplete; also, the parathyroid was preserved. In the cartilage-fat transplant the fat tissue was fairly well preserved but there was here some increase in fibrous tissue and there were collections of lymphocytes. Parts of the transplanted muscle tissue were preserved and embedded in fibrous tissue.

In an additional series of experiments we exchanged thyroid, cartilage and fat tissue, with or without bone or muscle tissue, between non-inbred mice and inbred mice belonging to strains D, C57 and A. In these experiments the grades also varied as a rule between 1 and 2—; in a few cases, slightly better grades (2/2—) were obtained. The reactions were usually more severe after 20 than after 12 days. Occasionally there was some lymphocytic infiltration in the thyroid transplants; in the fat tissue there was partial invasion by connective tissue, vacuolated phagocytic cells and lymphocytes.

Among the many experiments in which tissues were exchanged between different strains of inbred mice, we may mention one set in particular, in which thyroid, cartilage and fat tissue, with associated tissues, as well as pieces of striated muscle or ovary were transplanted into each host and examination took place after 20 days. In ten transplantations to different hosts the grades varied in the individual cases between 1 and 2—. When grade 1 was given, only the cartilage or parts of cartilage were preserved, but perichondrial regeneration of cartilage could take place around necrotic areas. The fat tissue as a rule was, to a variable extent, invaded by small vacuolated phagocytic cells and by connective tissue; infiltration with lymphocytes varied in different cases; also the amount of preserved fat tissue was variable, but on the average, the amount was greater in these transplantations in mice than in rats and guinea pigs. There were also some collections of polymorphonuclear leucocytes, especially in the fat tissue, and more prominently around fat cells which were enclosed in fibrous tissue. The thyroid was either entirely replaced by

fibrous tissue in cases in which grade 1 was given, or variable parts were preserved; in the latter case, the transplant was stunted, even if an almost complete chain of acini was found in a fibrous nodule. Lymphocytes could be lacking in such grafts, but in other instances some collections of lymphocytes were found in certain places; the dense masses of lymphocytes, which occurred so often in rat and guinea pig, were as a rule absent in the mouse. The transplanted striated muscle was either wholly necrotic or small numbers of regenerated muscle fibers filled with nuclear chains could be seen. In the muscle likewise, some lymphocytes could accumulate. The average grade in these ten transplantations corresponded to 1+.

We have carried out in addition, several other large series of experiments, in which at different times, extending over a number of years, we determined the mode of reaction in the reciprocal exchange of tissues between the following inbred strains of mice: A, D, C3H, CBA, C57, Old Buffalo, New Buffalo, C, and AKA. A detailed discussion of these experiments will not be undertaken, but a brief statement of the principal results may be made. The examination took place, as a rule, between 12 and 30 days following transplantation; injurious effects, on the average, increased with increasing time of exposure to the bodyfluids and cells of the host. The grades were changed correspondingly. After 20 and 30 days, they varied in the majority of cases between 1 and 2− ; but in some cases the grades were slightly higher than 2−, without however definitely reaching 2. Intra-strain transplantations, which were carried out at the same time, yielded higher grades. There was quite generally a correspondence between the state of preservation or injury of the various tissues in individual experiments. However, this did not necessarily involve a correspondence in the degree of lymphocytic infiltration, because the latter was often determined by local factors, among which, perhaps, local infection with bacteria played a role in a number of cases. While lymphocytes were by no means present in all homoiotransplants of cartilage and fat tissue, some increase in connective tissue and infiltration of the fat tissue with small vacuolated phagocytic cells was the most frequent indication of the incompatibility between the homoiogenous hosts and transplants. The lymphocytic infiltration cannot serve, therefore, as an indicator of the relationship between the individuality differentials of host and donor in the mouse to the same extent as in guinea pig and rat.

We have, thus, in these inter-strain transplantations, to deal with marked homoio-reactions similar to those found in transplantations of homoiogenous tissues in rats and guinea pigs. They differ from the latter in the decidedly decreased invasion of the grafts by lymphocytes and by connective tissue, in the frequent preservation of a stunted thyroid, in which lymphocytic infiltration was absent, and in the usually much diminished organizing activity of the connective tissue. As in the case of the rat, so also in the mouse the muscle fibers which were transplanted with xiphoid cartilage and fat tissue were relatively more resistant than the bone marrow, which was invariably destroyed in these homoiotransplants. Also, in the mouse the perichondrium was able to regenerate new cartilage, but the connective tissue cells seemed

to penetrate less readily into necrotic areas in the cartilage than in the rat. On the whole, it is evident that in the mouse the injury and destruction of homoiogenous tissues by the bodyfluids preponderate over the damage inflicted by lymphocytes and connective tissue, and that the activity of the latter may or may not be added to the action of the homoiotoxins of the circulating bodyfluids.

From our observations, it follows that transplantation of thyroid gland, cartilage and fat tissue, together with the associated tissues, cannot serve as accurately as an indicator of the relationship between the individuality differentials of host and transplant in mouse as in rat and guinea pig. It is advisable wherever possible to use, in addition to these transplants, grafts of ovaries and of striated muscle. A comparison of the effects of transplantation on a combination of these various organs may then serve as a good indicator of the degree of compatibility or incompatibility between the individuality differentials of host and donor.

Chapter 4

Autogenous, Syngenesious, Homoiogenous and Interracial Transplantations in Birds

In our experiments with Addison, in which we compared the homoiotransplantation of pigeon skin with the transplantation of this tissue into chickens, into various mammalian species, and also into amphibia, we found a marked difference between the results of homoio- and heterotransplantation. In the former, the lymphocytes of the host were the principal agent which injured and in the end destroyed the transplant, whereas, in the latter it was the toxicity of the bodyfluids which injured the transplants, caused a cessation of the proliferative power of the epidermis and, soon afterwards, destroyed it altogether. After heterotransplantation, this destruction was accomplished usually as early as during the first and second week, while after homoiotransplantation it took place in some cases during the fourth week, but in other cases transplants were found alive, at least partly, as late as during the fifth week.

While thus the distinction between homoiogenous and heterogenous transplants was quite sharp, and while there was also at least some indication that among the various types of heterotransplants there was, under certain conditions, a correspondence between compatibility of the organismal differentials and the degree of genetic relationship between the species, which served as hosts and donors, no attempt had been made in these experiments to analyze the finer differences in birds, which might be expected to exist between autogenous, syngenesious, homoiogenous and interracial transplantations. Nor did the subsequent experiments of Schultz, nor those of Danforth and Foster, give any information in this respect, although the latter in particular were of interest from other points of view. Danforth and Foster, in experiments with Leghorn and Plymouth Rock chickens, transplanted skin flaps from recently hatched chicks to other chicks of the same inbred race or to other races. In many cases the pieces of skin healed in permanently in chicks belonging to other races, although the best results were obtained in the exchange of skin between members of the same inbred race; but this may have been due to accidental factors rather than to a similarity of the organismal differentials between host and transplant. Danforth and Foster concluded that individuality differentials exist in birds in isolated instances. However, the fact that they used recently hatched chicks rather than adult birds made the recognition of differences between individuality differentials more difficult, because in these very young animals the reaction against strange individuality differentials should be milder or, under certain conditions, lacking altogether; in addition, in these long term experiments a gradual adaptation between host and graft might take place. Furthermore, the use of healing-in or lack of healing-in of

the skin flaps represents an "all or nothing" test, which cannot give any indication of intermediate results which might be found by means of a microscopic study of cellular reactions. Experiments in which we used microscopic studies of the cellular reactions against transplants in adult birds showed that there is no identity between individuality differentials even in brothers belonging to the same inbred race. Likewise, it may be found that in adult lizards homoiotransplantation of skin does not succeed, whereas autotransplantation is successful (May). By means of statistical analysis Kozelka found in skin grafts in Leghorn fowl, within the first few months after hatching, strong indications that the degree of relationship between donor and host, which signifies also the relationship between the individuality differentials of host and donor, is one of the factors which determines the success of the transplantations. Thus he found a persistence of grafts between unrelated birds in 18 per cent, between half and full brothers and sisters in 27 per cent, between full brothers and sisters in 50 per cent, and between offspring from father and daughter matings in 68 per cent of the transplantations. Similar to our experiences, he noted a correspondence in the behavior of several transplants from the same donor to the same host. However, also non-genetic factors, such as size or age of the donor and of the host, helped to determine the fate of the transplants. In accordance with expectation, transplantations between adult birds gave less favorable results than those between very young chicks, but in both instances the relationship between the individuality differentials of host and graft was the essential factor that determined the result of the transplantation.

In continuing our former experiments in birds, we made use first of inbred races of chickens, which we obtained from the Mount Hope Farm in Williamstown, Mass., through the kindness of Dr. Goodale. In these experiments we observed that notwithstanding close inbreeding, there was a marked lymphocytic reaction present, even around transplants in nearly related adult animals belonging to the same inbred race. This reaction was so strong that a definite and very distinct differentiation between the degree of similarity of individuality differentials in these animals seemed impossible and our investigations remained, therefore, unpublished. According to information given me by Dr. Goodale, these chickens had been inbred only for five or six generations of consecutive brother-sister matings. Likewise in our more recent experiments with guinea pigs, inbred for only a small number of generations of brother-sister matings, we did not yet observe a definite approximation of the individuality differentials in the various members of these families. We may therefore conclude that in order to achieve progress towards an autogenous constitution of the individuality differentials, a larger number of consecutive brother-sister matings is required than those which had been made in the chickens in Williamstown. Resuming these investigations more recently with W. J. Siebert, we confirmed the finding that also in the exchange of tissues between brothers of strains of chickens inbred to a limited extent, a very intensive lymphocytic infiltration and destruction of the transplants take place, and that syngenesio-, homoio-, and interracial transplantations in such chick-

ens all behave in about the same manner, although some very slight differences may exist. Thus while in homoio- and interracial transplantations the intensive lymphocytic infiltration set in about 10 to 11 days following transplantation, in syngenesiotransplantations it appeared a few days later, namely, after 13 days. Similarly, follicle-like accumulations of large lymphoblast-like cells, which were found in these grafts in chickens and which aided the smaller lymphocytes in the destruction of the strange tissues, were seen in the first two types of transplantations after 13 days, and in syngenesiotransplantations only after 16 days. While these differences in the time of the appearance of such cells are very small, still they are in agreement with the findings of H. T. Blumenthal in regard to differences in the time when the lymphocytes are increased in the circulating blood after subcutaneous transplantation of various pieces of tissue. It might be expected that the rapidity with which these changes in the lymphocytes and lymphoblast-like cells become manifest locally and the rapidity with which the increase in the lymphocytes takes place in the blood, should be greater in those cases where the individuality differentials, diffusing into the surrounding tissue or into the blood vessels, showed a greater degree of strangeness and therefore also a greater toxicity.

In contrast to these types of transplantations, after autogenous transplantation of skin and xiphoid cartilage with the surrounding tendon-like tissue, collections of lymphocytes are lacking altogether or only very small clumps of these cells, arranged around the vessels, can be seen. If keratin from the transplanted skin has been separated from the epidermis by the connective tissue, a few lymphocytes quite commonly collect around such foreign bodies. Lymphocytes are either absent or only very small collections form around particles of fat tissue transplanted with the cartilage or around some foreign bodies.

A very interesting occurrence is that sometimes around and in these autogenous transplants a disequilibration between the host connective tissue or the transplanted tendon connective tissue and the cartilage takes place. Then connective tissue cells move toward the piece of cartilage and surround it, giving rise to a capsule. Often they penetrate also into the periphery of the graft in the direction of the fibrillar structure of the long axis of the cartilage cells. In some cases, turning approximately at right angles to the long axis of the cartilage cells, they penetrate slightly into the interior of the cartilage. Moreover, these connective tissue cells possess the power to split and dissolve the cartilage, and in doing so, they sometimes become larger. Either in the cartilage or in the surrounding dense fibrous tissue some cells, coming from the connective tissue, may change into epithelioid and giant cells, especially in places where an obstacle interferes with their progress. Connective tissue cells also accompany certain vessels which grow into the cartilage. But on the whole, the transplanted cartilage, as well as autotransplanted fat tissue and bone marrow with myelocytes, is well preserved.

There is a remarkable correspondence between the reactions of the host connective tissue towards autotransplanted cartilage and towards autogenous epidermis transplants when the latter do not close to a cyst-like or to a flat

body. The keratin, and perhaps also remnants of feathers or other foreign material, can in these cases act as non-specific stimulators for the host fibroblasts, or even for the transplanted fibroblasts, and this process can sometimes lead to the destruction of the transplants; but in these instances, we have to deal with non-specific reactions of connective tissue cells and not with specific reactions on the part of lymphocytes induced by strange individuality differentials. The great difficulty on the part of the chicken and pigeon skin in forming a typical closed cyst, probably due to the low degree of growth intensity of the avian epidermal cells, is an obstacle to the successful subcutaneous transplantation of skin in this class of animals. But we must sharply distinguish between non-specific extraneous factors and specific factors which can be used in the analysis of individuality differentials. In the former, we have to deal with general tissue reactions, and these present important data which can be used in the construction of a physiology of tissues.

Various difficulties, then, are encountered, which limit a successful analysis of the individuality differentials in birds. They include: (1) the difficulty of obtaining the organs and tissues most suitable for transplantation in living animals; (2) the presence of complicating, non-specific factors which may cover up the reactions characteristic of the individuality differentials, as, for instance, the connective-tissue reactions which we have mentioned, and (3) the preponderance of lymphocytes in the circulation of birds, with which is associated an excessively strong reaction of lymphocytes against even very slight differences in individuality differentials; the great intensity of this reaction makes the discernment of smaller differences in individuality differentials difficult. However, the experiments which we have discussed do prove the presence of very fine differences in individuality differentials in birds, inasmuch as they have shown that such differences exist also between brothers in inbred strains or races of birds.

However, the great intensity of the lymphocytic reaction in this class of animals makes possible the clearer recognition of the mode of action and of the effects of the infiltration of a tissue with large masses of lymphocytes. In the experiments with chickens it could be seen that these cells are able to cause the disintegration of such structures as tendon-like fibrous tissue, cartilage, and even bone, resulting either in their complete solution or at least very extensive destruction, and leaving behind a fine network of remnants of these tissues. In mammalian transplantations it has not been possible to observe such marked effects; this is due presumably to the usually less massive invasion of mammalian tissues by lymphocytes, even in cases in which lymphocytes form lymph-gland-like accumulations in the tissues, such as we found especially in rat and guinea pig. It appears doubtful whether mammalian and avian lymphocytes otherwise differ markedly in their destructive power on tissue, which is presumably due to the action of enzymes. In the mouse, for instance, one can see in homoiogenous transplantations that lymphocytes as well as connective tissue cells are able to penetrate into hyaline connective tissue with ameboid processes; they move, in the latter, in the direction of the fibers and lymphocytes may likewise invade pieces of cartilage in the direction

of the preformed fibrillations of this tissue; but under such unfavorable conditions these cells may later perish.

The effect of the individuality differentials of autogenous, syngenesious and homoiogenous transplants on the lymphocytes in the circulating blood. Disharmony between the individuality differentials of the transplant and host not only causes local reactions, owing to the diffusion of the individuality differential substances into the area directly surrounding the transplant, but according to the findings of Blumenthal, diffusion takes place also into the blood vessels, and probably into the lymph vessels, from which points these differentials are carried presumably to the blood-forming organs and here stimulate an increased production or elimination of the white blood cells into the capillaries. This occurs in birds as well as in mammals. There is, moreover, a quantitative relation between the kinds of individuality differential substances given off by the transplants and the kind and intensity of changes induced. It may furthermore be assumed that even in the case of the local reactions around the transplants these substances diffuse into the blood or lymph stream and thereby contribute to the local accumulation of the white blood cells; at least the filling of the lymph vessels with lymphocytes and the increased number of the latter in the blood capillaries around homoiotransplants suggest such a process.

Investigation of the effect of homoiogenous and syngenesiotransplants by Blumenthal gave the following results: A relative and absolute increase in the number of lymphocytes in the peripheral blood took place after homoio- and syngenesiotransplantation, in contrast to heterotransplantation, after which an increase in polymorphonuclear leucocytes occurred. The general and local reactions were found at about the same time. After homoiotransplantation of the thyroid gland the maximum in the increase in lymphocytes in the blood was on about the 6th or 7th day; this was also the time when the lymphocytes began to collect locally around the graft; on the other hand, the maximum increase in the polymorphonuclear leucocytes after heterotransplantation occurred more rapidly, namely, on the 4th or 5th day. The time when the maximum in the response was attained depended also upon the consistency of the different tissues; this factor seemed to determine the readiness with which these specific substances, possessing the individuality differentials, were extracted and were able to diffuse into the adjoining areas and into the circulation of the host. The relative increase in the percentage of lymphocytes after homoiotransplantation of thyroid varied from 13.5 per cent in the pigeon, to 16.9 per cent in the rat, and to 16.6 per cent in the guinea pig, and in a number of experiments it exceeded 25 per cent. After heterotransplantation the relative increase in the percentage of polymorphonuclear leucocytes ranged between about 12 per cent and 26 per cent. After autogenous transplantation of different organs or tissues, the average maximum percentage increase of lymphocytes showed variations between 3.3 and 6.5 per cent in different species of animals; in the pigeon there was no increase. After subcutaneous transplantation of inert foreign bodies or after an incision the effect on the number of the circulating lymphocytes was about the same as after autogenous transplanta-

body. The keratin, and perhaps also remnants of feathers or other foreign material, can in these cases act as non-specific stimulators for the host fibroblasts, or even for the transplanted fibroblasts, and this process can sometimes lead to the destruction of the transplants; but in these instances, we have to deal with non-specific reactions of connective tissue cells and not with specific reactions on the part of lymphocytes induced by strange individuality differentials. The great difficulty on the part of the chicken and pigeon skin in forming a typical closed cyst, *probably due to the low degree of growth* intensity of the avian epidermal cells, is an obstacle to the successful subcutaneous transplantation of skin in this class of animals. But we must sharply distinguish between non-specific extraneous factors and specific factors which can be used in the analysis of individuality differentials. In the former, we have to deal with general tissue reactions, and these present important data which can be used in the construction of a physiology of tissues.

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tissue; or it may represent a rudimentary heterogenous reaction, in which the as yet imperfectly developed organismal differential is able to activate the lymphocytes but is not yet strong enough to act on the polymorphonuclear leucocytes.

In general, we may conclude from these data that the method used by Blumenthal is a very useful one if we wish to obtain comparable data of a quantitative nature and that in this respect it surpasses the use of the local reactions. But the latter give an insight into the effect of various types of individuality differentials on different kinds of host cells; it makes possible, furthermore, the differentiation between the effects of the bodyfluids and of the host cells on the transplant. There is, in addition, the possibility that the reactions in the circulating blood are complicated by non-specific or less specific substances which may be present in the transplanted tissues. Thus Blumenthal found that also the introduction of certain non-living protein substances into the subcutaneous tissue may cause an increase of lymphocytes in the blood, similar to that noted after the introduction of living tissues. However, in this case heating the protein substances does not lead to a loss of the general lymphocytic reaction as does the heating of the tissues. Moreover, at least as far as we know at present, the fine shading of the reactions in accordance with the relationship between host and transplant, which is characteristic of the introduction of pieces of living tissue, is lacking in the case of the protein material. These complications are apparently absent in the case of the local reaction. Considering all these facts, it seems that the combined use of these two methods is preferable to the application of either of them alone.

tion. The conclusion seems therefore justified that the increase after transplantations in which host and donor are identical is a non-specific reaction.

Syngenesiotransplantation of the thyroid in guinea pigs caused an average maximum percentage increase of lymphocytes amounting to 11.7 and the average period of the maximum increase was 12.1 days after transplantation, as compared with the corresponding figures in case of homoiotransplantation of the guinea pig thyroid, which were respectively 16.6 per cent and 7.1 days. The increase in the lymphocytes after syngenesiotransplantation was therefore less than after homoiotransplantation and it appears later, indicating a milder reaction in the former, a fact which harmonizes with the decreased disharmony of the individuality differentials between nearly related individuals as compared to not nearly related, homoiogenous individuals.

The conclusion that relationship between the individuality differentials of host and donor is the decisive factor in the changes in the distribution of white blood cells following various types of transplantation agrees also with the results obtained in mice. After transplantation of tissues to a different strain, the average lymphocytic increase was 17.7 per cent, and after transplantation within the strain it was 12.1 per cent. However, after transplantation within strain A, the most closely inbred strain, the increase in the lymphocytes was only 10 per cent and the maximum increase appeared somewhat later, on the average, after 13.6 days. These transplantations correspond therefore to the syngenesiotransplantations in the guinea pig. The response of strain A mice to heterogenous transplants from the rat was similar to that seen in other types of heterogenous transplants; the average increase in polymorphonuclear leucocytes was 18.7 per cent; it set in between the 2nd and 4th day and, as usual, was followed by a secondary rise in lymphocytes. There was not only a relative increase in the lymphocytes or leucocytes in the various types of transplantations, but also an absolute increase, which was even more striking than the relative increase. In the guinea pig the absolute increase in the number of lymphocytes amounted to about 78 per cent and in the rat to about 54 per cent, while the average increase in polymorphonuclear leucocytes reached about 110 per cent.

This method of analyzing the individuality differentials lent itself well to a comparison between the individuality differentials in adult and in embryonal tissues. It was found that tissues obtained from fully developed embryos near the time of labor behaved after homoiogenous and heterogenous transplantation in the same way as the corresponding adult tissues. On the other hand, very young embryonic tissues removed from an animal at about the conclusion of the first third of pregnancy behaved differently; here, after heterogenous, as well as after homoiogenous transplantation, a lymphocytic response similar to that noted after homoiogenous transplantation was observed, while an increase in the number of polymorphonuclear leucocytes in the host was lacking under these conditions. This indicates that the typical heterogenous organismal differentials had not yet developed in these cases. But the lymphocytic reaction may correspond to the increase in lymphocytes in the blood noted after transplantation of various dead protein substances into the subcutaneous

possessing strange individuality differentials and injure them; but they do so to a very different degree in different cases. The effect may be so slight that it is hardly noticeable; but in other cases the direct injurious action of these substances is quite marked and in different species the relative preponderance of the influence of the host cells and of the bodyfluids varies, the latter being relatively more important in the mouse than in the guinea pig and rat. The fact that an interaction between transplanted tissues and the bodyfluids of the host takes place in every instance makes it difficult to decide whether the host cells are activated by the homoiogenous individuality differential of the transplant directly, or only after the latter has combined with the homoiotoxins of the host.

The growth processes, and in particular the mitotic cell multiplication, which occur in the transplanted tissues are not entirely regenerative in character, but they may be due partly to the continued function of a primary tendency to mitotic proliferation, which is inherent to a very different degree in different tissues. In tissues in which secondary differentiations have taken place, as for instance, in cartilage and striated muscle tissue, or in epidermal cells at some distance from the source of oxygen supply, the tendency to undergo mitotic proliferation is replaced by amitotic processes. Certain unfavorable environmental factors may likewise prevent mitotic proliferation and instead cause formation of epithelioid and giant cells, and in general favor processes of differentiation instead of mitotic proliferation.

2. *The mechanism which leads to the specific reactions of the lymphocytes of the host against the transplant.* We have seen that different homoiogenous tissues may attract the lymphocytes to a different degree, and we shall report on particularly striking instances of such differences between different organs in subsequent chapters when we discuss the transplantation of adrenal gland and anterior hypophysis in mice. In addition, it was possible to demonstrate, in the rat, the attraction which homoiogenous tissues exert on the lymphocytes of a nearby lymph gland of the host, in experiments which Crossen carried out in the guinea pig. He autotransplanted a lymph gland into the subcutaneous tissue and then placed either a piece of autogenous or homoiogenous xiphoid cartilage near the lymph gland, or into the lymph gland itself. While the lymphocytes of the transplanted lymph gland were inactive towards the autogenous cartilage graft, they were actively attracted by the homoiogenous tissue and they migrated into the homoiogenous transplant. This may be considered as confirmatory evidence for the conclusion that the movement of the lymphocytes towards the homoiogenous transplants represents a chemotropic reaction.

3. *Differences in the intensity of the reaction against strange individuality differentials observed in different families or strains of rats.* If tissues are homoiotransplanted from certain families or strains of rats into other families or strains, different average degrees of severity in the reactions may be observed. We have analyzed the factors which cause these differences in several series of experiments carried out in rats. For this purpose we compared the reactions in rats from various strains and families, obtained from different

Chapter 5

The Mechanism of the Reactions Against Homoiogenous Individuality Differentials; Autogenous Tissue Regulators

1. *Various phases which follow auto and homoio transplantation.* It follows from the observations discussed in the preceding chapters, that after transplantation there is a first phase in which there is no noticeable difference between the conditions of autogenous and homoiogenous transplants. This phase is dominated by the injury due to the process of transplantation and by injurious conditions existing in the new location of the tissue. The damage to the tissues is followed by regenerative reactions; the homoiogenous tissues are subjected subsequently, during the second phase, to further specific injuries by the host and these may also call forth regenerative processes as long as the injury has not progressed too far. These injuries, furthermore, initiate the activity of the host connective tissue, which moves towards the transplants. There originate, thus, general, partly non-specific changes, which are based on attributes of the grafted tissues and of the host tissues.

This first phase is followed by a second one, in which differences develop between the autogenous and the homoiogenous transplanted tissues. There is a preponderance of regenerative growth processes and regulative processss in the autogenous transplants, and there are injurious effects which the host exerts on the graft under the influence of homoiogenous individuality differentials. The latter tend to prevent a satisfactory recovery of the transplanted homoiogenous tissue from the injuries received during the first phase, and they cause additional damage to the homoiogenous transplant, which thus, in many cases, cannot maintain itself and during the third phase is gradually destroyed. These are the characteristic features of the second and third phases following transplantation, in which differences between the conditions of the autogenous and homoiogenous tissues become more and more marked. However, there occur, also, changes opposed to this outcome, namely, conditions of adaptation between transplant and host, which in certain instances may slowly lead to an improvement in the state of the homoiogenous transplant and may make possible its survival in the strange host.

As to the mechanisms leading to the secondary injury of the homoiogenous tissue, they consist, in the first place, in the action of the homoiotoxins of the host, and secondly, in the activities of the host cells; the most specific among the latter are the reactions of the lymphocytes; but also the behavior of the connective tissue and blood vessels is influenced by the homoiogenous character of the individuality differentials. Furthermore, the age of the host influences the action of the connective tissue; the latter is diminished if the host is very young. It seems that in every instance the homoiotoxins act on tissues

tions likewise, as a rule, were constant in pieces of tissue from the same donor, transplanted into the same host, provided we consider that there are various variable factors which complicate such experiments and, in particular, that as a rule only in transplants in which there is a large amount of living tissue left is the lymphocytic infiltration considerable.

However, there were strong indications that, in addition, another factor played a role in certain instances. Thus we observed that in the same host the reactions against various tissues from two different homoiogenous donors, while not necessarily identical, were correlated with each other. Furthermore, if tissues from two donors were each transplanted into two different hosts, the reactions against both could be severe in one host and relatively mild in the other host. It was especially the Bu rats which, in almost all cases, reacted very severely against transplants from other families or strains of rats, the grade being 1 in the large majority of cases, while the reciprocal transplantations, in which Bu rats were the donors and other families the hosts, gave a much greater number of milder reactions. The Bu rats, before being used for transplantation, had been fed for some time on a riboflavin-deficient diet; but that this was not the essential cause of the strong reaction against strange individuality differentials which these rats exhibited was shown in control experiments, in which this same strain of rats had always been kept on a normal diet, but the severity of the reaction was not diminished; nor was the age (weight) of the animals used, nor the season of the year when the experiments were carried out of significance in this respect. Only in a single experiment in which these rats served as hosts were the reactions somewhat milder. While thus the Bu rats reacted in almost all instances very strongly against homoiogenous differentials, the homoiogenous differentials of Wistar rats, serving as donors, seemed to elicit less strong reactions on the part of the host than did some of the other strains, although they still remained within the homoiogenous range. There is, then, some strong experimental evidence for the conclusion that certain strains of rats, and probably also other species, have the peculiarity of reacting especially strongly against strange individuality differentials, and it is furthermore possible that the grafted tissues from certain strains of rats, acting as donors, stimulate the host cells less actively than do the tissues from other strains. A second set of factors exists therefore besides the degree of strangeness between the individuality differentials of host and transplant, which determines the severity of the reaction of the host against the transplant, namely, a peculiar reactivity of the host tissues which presumably has also a genetic basis. There is, besides, some evidence that not only strains of animals have this peculiarity in their mode of reaction, but that also various individuals may differ from one another in this respect.

4. *The effect of heat on the homoiogenous individuality differentials in rats.* In these experiments we subjected thyroid and cartilage-fat tissue of Bu rats to boiling temperatures for 5 minutes and then transplanted the pieces into Chicago rats. Under these conditions the homoiotransplanted tissues were entirely necrotic. After 12 days, the nuclei in the thyroid and parathyroid were found dissolved in the peripheral, and shrunken in the central acini.

breeders in different cities. Various combinations of donors and hosts were tested, and in a number of experiments tissues from one donor were placed into the left side, and those from another donor into the right side of a host; in other cases, pieces of tissues from the same donor were transplanted into two different hosts. In the majority of these experiments the examinations took place 20 days after transplantation.

In order to compare the intensity of the reaction in different combinations of hosts and transplants it is necessary to make equal the times at which the examination takes place. With increasing time, the severity of the reactions as a rule increases. If we consider all these experiments together, we may conclude that autotransplantation, where the individuality differentials of host and transplants are identical, reactions which do occur are due to injury inflicted on the grafted tissue during the operation or to the abnormal conditions under which the transplants live in their new environment, and that these abnormal conditions are, as a rule, overcome in the course of time. On the other hand, in the case of homoiotransplantations the reactions are caused by the differences in the individuality differentials between host and transplants, and in different combinations of families or strains the severity of the reaction in the host and the injury in the donor differ. While in some combinations the reactions are severe, as indicated by grade 1, in others the grades range between 1 and 2—; in still others the average grade may be 2—, or even somewhat higher, and in rare instances, grade 3—, or even 3, may be reached in an animal. These grades apply only for a certain length of time, during which the transplant was exposed to the influence of the host; this period was 20 days in this series of experiments and there are indications that after 30 or 40 days the reactions would have been more severe and the grades accordingly lower. Essentially two factors are responsible for the grades thus obtained. In the first place, these differences in the reactions are due to differences in the relationship between the individuality differentials of host and donor. This is indicated by the fact that if various tissues are transplanted from one donor into the same host, the severity of the reaction is the same in all the pieces, if we make allowance for the peculiarities which distinguish different types of tissues. This conclusion harmonizes with the many other transplantations which we have carried out with homoiogenous tissues. Thus, in a certain experiment in which grade 3 had been given, a great portion of the thyroid transplant was preserved; in the muscle transplant there were long parallel muscle fibers with good cross-striations, and in one specimen of this kind even a mitosis seemed to be present in a muscle cell. Likewise, the grafted fat tissue was well preserved. With grade 2 there was much lymphocytic infiltration in thyroid and muscle and at least a large part of the fat tissue was preserved, while with grade 1, neither thyroid nor muscle was preserved and the fat tissue was mostly replaced by connective tissue, small vacuolated cells and lymphocytes. Furthermore, it could be seen that the individuality differentials of both host and donor determined the intensity of the reactions; this follows from experiments in which either the donor or the host varied, while the other partner which entered into the combination remained constant. The lymphocytic reac-

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between tissues and it is based essentially on inherited properties of the tissues. These inherited characteristics assert themselves in all instances, irrespective of the autogenous or homoiogenous state of the transplants. In certain tissues, which possess great resistance to injurious conditions and in which the regenerative momentum is very strong, regenerative processes may take place in a homoiogenous medium, but the homoiogenous relationship always tends to act as an injurious factor, preventing or at least inhibiting regenerative activities.

There enter, then, at least two factors in the creation and maintenance of the autogenous tissue equilibrium, namely (1) the individuality differentials, which diffuse from the tissues into the surrounding bodyfluids and which are present also in the blood; (2) other factors inherent in the tissues, which assert themselves under certain conditions, as, for instance during regeneration. There exists the probability that the autogenous individuality differentials as such function as these tissue equilibrizing substances; but it is also conceivable that there may exist special autogenous tissue-regulating substances, which possess a chemical group characteristic of the individuality differential of the host, or that there may be separate autogenous substances devoid of the individuality differential but which could induce the tissues to react in the normal manner only in an autogenous medium. However, the first interpretation seems to be the simpler and more probable one. Just as the homoiogenous individuality differential exerts abnormal effects on various types of cells, so the autogenous individuality differential may be expected to exert the opposite functions, which in contrast to the homoiogenous substances bring about and maintain a normal relation between the various tissues. These substances, acting on adjoining tissues as contact substances which latter in a wider sense may be included among the hormones, although not usually thus classified, and acting also on distant tissues as hormones in the usual meaning of this term, would thus possess a very important function in making possible the harmonious interaction of the many constituent parts of the individual organism; however, they would be supported in this task by other typical hormones produced in distant organs, which do not necessarily possess the individuality differential, and also by specific elements of the nervous system.

Fibrous tissue grew around and into the transplant. Also, cartilage and fat tissue were necrotic, but the peripheral portions of these pieces had been fixed as a result of the boiling. Connective tissue and small vacuolated cells grew into the fat tissue, but the lymphocytic reaction was lacking. After 20 days, the results were similar. In the thyroid, some balls of hard colloid, surrounded by some giant cells and fibrous tissue, were seen. At this time also, connective tissue grew into thyroid and into necrotic fat tissue, in which, in addition, small vacuolated and epithelioid cells were seen. A few polymorphonuclear leucocytes were likewise noted in the connective tissue, but a typical lymphocytic reaction was absent, and the homoiogenous fat tissue did not attract polymorphonuclear leucocytes. These experiments confirm, then, the conclusion that in general, if we except reactions of a non-specific nature, such as the ones elicited by certain dead foreign bodies, only living tissue calls forth the lymphocytic reaction which is characteristic of transplants possessing homoiogenous individuality differentials.

5. *The autogenous tissue regulators.* In the normal organism the various types of cells have inherited those modes of interaction with other cells and intercellular substances of different kinds, by means of which the mutual cell and tissue relations are safeguarded. Hence, whenever the various types of transplanted tissues possess the same autogenous chemical characteristics of the individuality differential as the host, they tend to interact with the host tissues as if they were a normal constituent of the host, even if at first a disorganization has taken place as the result of temporary accidental conditions. The disappearance at later dates of factors disturbing the normal tissue relations directly following autogenous transplantation of thyroid, cartilage, fat tissue and uterus, indicates the presence of autogenous regulators. However, not in all organs do such regulators suffice to overcome the abnormalities brought about by the operation; for instance, in the transplants of kidney a complete normality in the structure of the transplant does not need to be achieved. A perfectly closed epithelial layer may survive permanently after autotransplantation, but incompletely closed epithelial structures, such as kidney tubules which have been cut at one end, and especially the more differentiated convoluted tubules or epidermal cysts which are interrupted by hairs, are at a disadvantage and may die even after autogenous transplantation.

After homoiogenous transplantation of various tissues and organs in a number of species, the interaction of disharmonious individuality differentials leads to abnormal relations between host cells and transplanted tissue. The homoiogenous substances given off by the transplants stimulate and attract lymphocytes and connective tissue cells, with graded intensities which exceed the threshold of normality, and in addition, homoiogenous substances of the host may injure directly the homoiogenous tissues, whose relation to stroma cells and blood vessels and to lymphocytes in the adjoining areas of the host is thus altered. In the end, in the large majority of cases the transplanted tissue is either destroyed or at least its normal structure and relations to the neighboring tissues are not completely re-established.

Regeneration may also be considered as a regulatory process in the relations

should pass from the zone of syngenesio-relationship and reactions to the autogenous zone; but in reality this does not seem to be fully accomplished. A closely inbred strain is a strain of brothers and sisters, which are very similar in genetic constitution.

After these introductory remarks, we shall consider the results of syngenesiotransplantation in the rat and in the guinea pig, and then we shall analyze the interaction of the individuality differentials observed in transplantations in closely inbred strains of rats, guinea pigs and mice.

(a) *Syngenesiotransplantation in rats.* We have referred already to some experiments in which the reactions against transplants from brothers or sisters were, on the average, milder than the reactions against homoiogenous transplants, but on the other hand there were some instances in which no sharp distinctions between the individuality differentials of brothers and sisters and those of not closely related individuals could be established. Thus in the mutant Wistar rats, a special strain developed by Dr. Helen Dean King, the syngenesio-reactions were milder than the reactions against transplants from non-related rats of the same strain, if the examination of the grafts occurred 12 and 16 days after transplantation, but no difference was found after 20 days. Also in some other experiments the grades in syngenesiotransplantations could approach closely the average grades in homoiotransplantations, although their average reaction was still somewhat milder.

Two series of experiments will now be discussed—one made in 1918 (series I), and the second made in 1927 (series II), in which we compared the fate of various organs such as skin, ovary, uterus, spleen, liver and thyroid after various types of syngenesiotransplantation and of homoiotransplantation. More than one organ, as a rule, was transplanted into each host. The average grades obtained in these series are given in the following table I.

TABLE I

	Series I	Series II
Autogenous transplantation	3.15	
Homoiogenous transplantation	1.24	Variations between 1 and 1.75
Brother or sister to brother or sister	2.08	2.50
Parents to children	2.28	2.06
Children to parents	2.11	2.25
Grandparents to grandchildren		2
Grandchildren to grandparents		2.60

In the second series it was thought unnecessary to carry out autogenous transplantations, because these did not vary significantly in different experiments. In both series the grades were better in the various kinds of syngenesiotransplantations than in homoiogenous transplantations, and intermediate between those obtained in autogenous and homoiogenous transplantations. As to various types of syngenesiotransplantations, no consistent differences were found and those that were noted were not of the same kind in the first and second series.

Chapter 6

Syngenesiotransplantation, Transplantation in Closely Inbred Strains, and the Individuality Differentials of Near Relatives

The average genetic relationship between near relatives, such as brothers and sisters, parents and children, should be somewhere intermediate between the homoiogenous and autogenous relationship, and, accordingly, the average results of syngenesiotransplantation should likewise be somewhere intermediate between those of autogenous and homoiogenous transplantation. That this is the case is indicated by some experiments to which we have already referred. However, there may be instances in which such intermediate results are not evident, but in which the reactions obtained in syngenesiotransplantation cannot be distinguished sharply from those obtained in homoiogenous transplantation. Several conditions might account for this occurrence: (1) It might be due to the fact that even in syngenesiorelationship there may be such a degree of genetic difference between donor and host of the transplant that the threshold determining a reaction characteristic of a homoiogenous transplantation has been reached, although the individuality differentials of donor and host actually are more nearly related than is the case in the average of homoiogenous individuals. (2) It might also be due to the fact that when the threshold determining the homoiogenous reaction is very close to the autogenous region in the spectrum of reactions, the host cells are extremely active and efficient in discovering differences in genetic relationship and therefore the transplants, whose individuality differentials deviated only slightly from those of the host, are attacked with a maximum intensity, a type of reaction which we have found to obtain in birds. In those instances in which the individuality differentials of host and donor in ordinary syngenesiotransplantation are so far removed from each other that a mitigated reaction can not be demonstrated, an experimental intensification of the brother-sister relationship, through inbreeding, may make the genetic relationship closer than it is in ordinary brother-sister relationship. In this event the threshold point separating homoiogenous and syngenesious reactions may not yet have been passed and the difference in the reaction of the host against the tissues from a brother and from a not so nearly related individual belonging to the same inbred family or strain may then become manifest. In all essential respects, transplantations in closely inbred strains represent intensified brother-and-sister relationships, because the closely inbred strains were obtained by brother-sister matings in consecutive generations. Theoretically, it would be expected that after a certain number of consecutive brother-sister matings have been made, the relationships even between individuals in the inbred strain other than brothers and sisters,

intensities of damage in different tissues, and they affect these tissues, therefore, in a graded manner. In general, only structures with an intermediate degree of sensitiveness are suitable indicators in the analysis of the individuality differentials. Tissues, such as cartilage, which are so little sensitive that they react in about the same way after autogenous, syngenesious and homoiogenous transplantation, are not suitable for this purpose. Likewise, tissues which are so sensitive that they are entirely or largely destroyed by the non-specific injury connected with and following the process of transplantation, such as adult testicle and brain of adult mammals, are not suitable test objects.

It is the simple constituents of various organs, those less differentiated as to structure and function, which are usually more resistant and tend to survive even if the conditions following transplantation are injurious. Unfavorable conditions of nourishment, such as deficiency in oxygen, may cause the differentiation of tissues—for instance, in the epidermis, in placentoma, in the large follicles of the ovary—and differentiation may result in both increased sensitiveness and a diminution or absence of proliferation, or in a proliferation of an abnormal kind, in which mitoses are lacking and, instead, amitotic nuclear multiplications occur; a production of epithelioid and giant cells, and hypertrophy rather than hyperplasia, are then characteristic findings in this condition. The most sensitive structures perish often after homoiotransplantation but may remain alive after syngenesiotransplantation. We may now briefly compare the relative sensitiveness of the various organ constituents which we have used for transplantation in the rat and classify them approximately in accordance with the effect which the different types of individuality differentials have on these constituents.

(1) *Skin:* In homoiogenous and syngenesious transplantation into the subcutaneous tissue the skin, which here forms a cyst, is usually destroyed, notwithstanding the fact that the epidermis as such is resistant. This destruction takes place because in the skin, after transplantation, the injurious action of the connective tissue elements is stimulated and strenghened in a non-specific manner. It is especially the loss of the epithelial lining of the hair follicles which may lead to the stereotropic ingrowth of the connective tissue and the destruction of the cyst, even in autotransplantation. Furthermore, under unfavorable conditions the whole epidermis of the cyst may become keratinized, owing to insufficient nourishment or to mechanical pressure by hyaline connective tissue, which may at times fill the cyst. Giant cells form around the hair and the keratin particles. This non-specific action of the connective tissue, and sometimes also of the lymphocytes, in co-operation with the homoio-, or syngenesio-toxins of the host, usually leads to the destruction of the epidermis after subcutaneous transplantation, owing to a summation of these partly non-specific and partly specific effects. In some instances, however, the action of the non-specific factors alone may lead to the destruction even of the autogenous skin, although in other cases this may remain alive for a long time or perhaps permanently. Mitoses are usually found in the hair follicles, which are more protected than other structures.

(2) *Ovary:* The various constituents of the ovary show a graded power of

In earlier investigations, Schoene compared various types of transplantations of skin into defects in the skin; he used as criterion of the results the healing in or the casting off of the transplants, which represents an all or nothing effect and does not allow the recognition of intermediate degrees of reactions. In young rats, autotransplantations of skin succeeded almost invariably, while in older rats the pieces were entirely or partly cast off. In transplantations between relatives, the most favorable results were obtained between brothers and sisters, provided the animals were young; but only in a small minority of cases did the grafts in relatives behave like autotransplants; in the large majority, they were cast off like homoiotransplants. However, altogether only eighteen transplantations between relatives were carried out, a number which, considering the method used and the results obtained, was hardly sufficient to differentiate between different types of transplantations.

If instead of considering merely the averages, also the intensity of the reactions in the individual experiments are taken into account, it is found that in the spectrum of relationships the grades of the syngenesiotransplants range between those characteristic of homoiogenous transplants and those approaching almost the grades characteristic of autogenous transplants, all transitions in grades being found. These results indicate the presence of multiple factors as the genetic determiners of the individuality differentials in the different animals. We have not to deal with the simple proportions of alternating Mendelian inheritance, such as we find if one or two factors are the hereditary determiners. The results are similar to those noted in the hereditary transmission of quantitative differences in the two parents, each quantity being represented by multiple factors and one-half of the multiple factors of each parent being united in the child; this condition would lead to a series of intermediate results in different matings and to the appearance of a blending inheritance. The combination of the multiple factors of the parents leads in the offspring to the production of a chemical substance, the individuality differential, which is present in all, or almost all, the tissues and organs of the child. The assumption of the presence of multiple factors as determiners of the individuality differential is also in accordance with the gradations in the intensity of the reactions against homoiogenous tissues which were found in the numerous experiments carried out.

The effect of variations in the individuality differentials of host and donor on transplanted organs and their constituent parts in the rat. We may here digress from the consideration of syngenesiotransplantations and discuss the manner in which different organs and tissues can be used in the analysis of the character of the individuality differentials and of the organismal differentials. Not only the reaction of the lymphocytes and of the connective tissue against the transplant, but also the survival and preservation of the constituent parts of the transplanted organs may be used in the standardization of the individuality differentials, provided their comparative power of resistance is taken into account, and, conversely, the various degrees of injury inflicted on these constituent parts of organs may serve as a test of their sensitiveness. Conditions prevailing in syngenesio-, homoio- and heterotransplantations cause different

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(1) *Skin:* In homoiogenous and syngenesious transplantation into the subcutaneous tissue the skin, which here forms a cyst, is usually destroyed, notwithstanding the fact that the epidermis as such is resistant. This destruction takes place because in the skin, after transplantation, the injurious action of the connective tissue elements is stimulated and strenghened in a non-specific manner. It is especially the loss of the epithelial lining of the hair follicles which may lead to the stereotropic ingrowth of the connective tissue and the destruction of the cyst, even in autotransplantation. Furthermore, under unfavorable conditions the whole epidermis of the cyst may become keratinized, owing to insufficient nourishment or to mechanical pressure by hyaline connective tissue, which may at times fill the cyst. Giant cells form around the hair and the keratin particles. This non-specific action of the connective tissue, and sometimes also of the lymphocytes, in co-operation with the homoio-, or syngenesio-toxins of the host, usually leads to the destruction of the epidermis after subcutaneous transplantation, owing to a summation of these partly non-specific and partly specific effects. In some instances, however, the action of the non-specific factors alone may lead to the destruction even of the autogenous skin, although in other cases this may remain alive for a long time or perhaps permanently. Mitoses are usually found in the hair follicles, which are more protected than other structures.

(2) *Ovary:* The various constituents of the ovary show a graded power of

resistance, which diminishes in the following order: (1) Interstitial gland, germinal epithelium and medullary ducts, both of which have a tendency to form cysts; (2) primordial and small follicles; (3) medium-sized and large follicles; (4) corpora lutea. For instance, six days after transplantation interstitial gland may be seen; it is derived from theca interna cells of atretic follicles, and perhaps also surviving parts of the granulosa may participate in its origin; these interstitial gland cells may give rise to larger cells, containing yellow pigment, which constitute or resemble interstitial gland cells and may act as phagocytes, taking up red blood cells in hemorrhagic areas and thus producing pigment. Under somewhat more favorable conditions of transplantation, primordial and small follicles may survive; and under still more favorable circumstances these small follicles may grow to medium-sized or large follicles. Under very favorable conditions of syngenesiotransplantation, the large follicles may rupture and give rise to corpora lutea; but the corpora lutea, consisting of differentiated cells, are sensitive and usually degeneration takes place if they are transplanted. In other instances the large follicles do not rupture but, instead, develop into large cysts. Ovulation in the transplanted ovary may occur synchronously with ovulation in the non-transplanted ovary of the host, perhaps in response to a hormone given off by the anterior hypophysis. In less than one-half of our transplants preserved, medium-sized or large follicles were found; this is a frequency which is somewhat less than that with which bile ducts were preserved in transplanted pieces of liver. If thyroid was transplanted simultaneously with the ovary, and if the reaction against the homoiogenous thyroid was severe, only the more resistant constituents of the ovary were preserved. On the other hand, in cases of a syngenesio-reaction, with grade 3 or 3—, in which therefore the individuality differentials of host and transplant were relatively harmonious, primordial and growing Graafian follicles as well as corpora lutea could be observed. However, lymphocytes infiltrated even better preserved ovarian transplants; they could appear first around vessels and then infiltrate also other structures, but only in rather rare instances did they infiltrate preserved follicles. Not only follicles, but even medullary ducts were found more frequently in syngenesio- than in homoiotransplantations. Around follicles, which after transplantation, underwent necrosis, giant cells could develop, which functioned as phagocytes and helped in the removal of the necrotic material. The removal of necrotic material proceeded very slowly and remained imperfect for a long time. On the whole there was, then, a great difference in the power of resistance of the various ovarian structures and there was a definite correlation between the types of transplantation and the kind of ovarian constituents which survived after transplantation; in general, the more resistant ovarian structures were less prone, whereas the most sensitive constituents of the ovary were more prone to injury than the acini in the thyroid gland transplanted simultaneously.

(3) *The Fallopian tubes and fimbria* belong to the most resistant and relatively best preserved organs, comparable in this respect to the more resistant constituents of the ovary and uterus and to the pelvis of the kidney; they

tended to survive even under homoiogenous conditions of transplantation, but were unfavorably affected by a marked disharmony of the homoiogenous individuality differentials.

(4) *The uterus* is, on the whole, also a resistant organ, although certain of its constituents may show less resistance. There was often necrotic material in the lumen of the uterus and part of its wall could be destroyed. The epithelium and the peritoneal endothelium were more resistant, while the unstriated muscle tissue was more readily injured after homoiogenous transplantation, and cellular, myxoid and predecidual connective tissue underneath the epithelial structures continued to live only if the individuality differentials of host and transplant manifested a high degree of compatibility.

(5) In *kidney* transplants the tubules and glomeruli, situated in the periphery of the transplant, were most prone to survive; the collecting tubules, with pelvis and ureter showing the least differentiation, were very resistant. On the other hand, the convoluted tubules were very sensitive to the injurious action of the unfavorable individuality differentials.

(6) After transplantation of pieces of *liver*, peripheral bile ducts remained alive in about fifty per cent of our transplants for 1 or 1½ months; they showed mitoses mainly in the earlier periods, as, for instance, 14 days after transplantation, but some mitoses were visible at later periods. The bile ducts corresponded, therefore, in their power of resistance to unfavorable individuality differentials, to the small follicles of the ovary or to spleen tissue. New bile ducts could develop and these structures were able to survive for as long as a month and a half, even under homoiogenous conditions, although they were better preserved after syngenesiotransplantation. In 38 per cent of the transplants in which bile ducts were preserved, or in a little more than on-sixth of all our transplantations of liver, liver cells as well survived, which is about the frequency with which megakaryocytes were preserved in the spleen. However, liver cells did not survive if the individuality differentials of the transplant were homoiogenous; but they could survive in favorable syngenesiotransplants, under conditions in which also mitoses were seen in the bile ducts. In certain instances, a liver cell with two nuclei was noted, but only in one case was a mitosis seen in such a cell. It may be remarked in this connection that under exceptional conditions mitoses may appear in young cartilage cells, or even in transplanted cells of striated muscle tissue. As in ovary and kidney transplants, so too in liver transplants the necrotic center could remain partly unorganized for a long time.

(7) *Spleen:* Between 36 and 47 days following transplantation spleen tissue was found preserved about as frequently as bile ducts in liver transplants or small follicles in ovarian grafts. Here, again, homoiotoxins proved injurious and the injury increased with increasing time following transplantation; eventually, only fibrous tissue with blood pigment was found. Syngenesiotransplants were more favorable and in these as well as in autogenous grafts Malphigian bodies, blood sinuses containing erythrocytes, mononuclear, phagocytic cells and trabeculae were seen, and at later stages megakaryocytes.

(8) *Testicle:* Testicle tubules as a rule perished, but a few peripheral ones,

lined merely with Sertoli cells, could survive. Testicle tubules were destroyed also after autogenous transplantation; this effect is therefore due to a non-specific injury, by which the more differentiated testicle cells are affected.

(9) *Striated muscle tissue* was relatively resistant to unfavorable individuality differentials, and even under the action of strong homoiotoxins some muscle fibers could survive and show amitotic nuclear proliferation.

(10) *Fat tissue*, at least in part, tended to survive even after homoiogenous transplantation; but it was readily invaded by connective tissue and by cells which acted as phagocytes, but which could in addition form giant cells, and in some cases, by lymphocytes. The tendency to invasion by these cells differed in different species; it was greatest in the guinea pig, where there was also the greatest tendency to the formation of giant cells, and it was least marked in the mouse; rat tissue, with which we are here more directly concerned, showed an intermediate position.

(11) *Bone, bone marrow and cartilage:* As stated previously, cartilage with the surrounding perichondrium is a very resistant tissue, which could survive and undergo regenerative growth processes even under very severe homoiogenous conditions. In bone, the bone cells tend to die, especially in the central parts, owing to a lack of nourishment. In the peripheral parts of transplanted bone it was often difficult to decide whether the cells situated here had come from the surrounding connective tissue, or whether they were actually preserved bone cells. Under certain conditions, new bone could be formed in transplants around the cartilage as well as in the bone marrow. The bone marrow, as a rule, survived only under very favorable conditions of syngenesiotransplantation.

In general, we may conclude that the results of transplantation of various tissues depend upon inner and outer factors, the former situated in the transplant and the latter in the host. Among the inner factors localized in the transplant, (a) the most prominent is the constitution of the individuality differential, which in its relation to the individuality differential of the host largely determines the fate of the transplant; (b) important too, is the degree of sensitiveness to injury or the power of resistance of the transplant or its various constituent parts to injurious conditions; and (c) also influencing the survival of the graft are certain accessory conditions, such as the presence of hyaline tissue or other resistant tissues in the transplant, which protect the more sensitive parts; the thickness of the transplant, which affects the size of the central, least nourished parts; these latter tend to die, while the peripheral parts remain alive. In addition, the age of the transplant may play a certain role, as well as its possession of a peculiar tissue constitution, which influences the activity of the lymphocytes of the host. Among the outer factors affecting the results of transplantation are the constitution of the individuality differentials of the host, the reactivity of the host against strange individuality differentials, and the presence in the host of immune substances directed against the transplant; besides, the place of transplantation may be of significance.

If we make allowance for the variations caused by all these factors, our experiments have shown that as a rule pieces of different tissues, transplanted

from the same donor into the same host, behave in the same manner and have a corresponding fate. There are hosts in which all pieces are well preserved, others in which all transplants are destroyed, and still others in which the transplants show an intermediate degree of preservation.

The behavior of lymphocytes towards different types of transplants. We have discussed non-specific factors which influence the fate of transplants, without any participation of lymphocytes or connective tissue cells being necessary in this process; we have also discussed non-specific reactions on the part of connective tissue towards the transplants. Likewise, in the case of the lymphocytes, factors other than the individuality differentials may be present in or around the transplant, and may influence the activity of these cells towards the graft. Thus foreign bodies around or in a transplant may cause an accumulation of lymphocytes, as may also epidermal cysts, in some instances even if the latter are autogenous in nature. Mildly inflammatory alterations of a chemical nature, or more severe ones acting at a distance from the exciting agent, may attract lymphocytes. But it seems that necrotic material does not exert a direct attraction on these cells; they do not usually invade necrotic material unless it is invaded first and organized by growing connective tissue; in the latter, some collections of lymphocytes are often found. But the most characteristic feature of the lymphocytes is that they are attracted by strange individuality differentials. The degree of this lymphocytic reaction shows a curve which has its maximum at a point intermediate between the autogenous and the severe homoiogenous zone of the relationship spectrum; the lymphocytic infiltration is frequently less marked in the latter zone, because here the greater part of the transplant has become either necrotic or is replaced by host tissue. The maximum of the curve may be in the syngenesious zone or in the zone of mild homoiogenous reactions. In the different types of transplantations lymphocytes move usually by way of the lymph vessels, but to a lesser degree, also by way of the blood vessels, in the direction towards and into the center of the transplant. To a certain extent they tend to accumulate in different places in different kinds of transplanted tissues. It is of special interest that they seem to prefer certain tissues to others. In the thyroid they may first collect along the inner border of the ring of preserved acinar tissue. In cartilage-fat transplants they accumulate in the fat tissue, where there is a deposit of fibrous tissue containing vessels; but they also collect around the perichondrium and cartilage and they may invade that portion of the cartilage which consists almost entirely of cartilage cells. In the skin they often avoid the sebaceous glands and the epithelium of the hair follicles when this is preserved. Of special interest is their behavior in the ovary; here they infiltrate first the fat and connective tissue surrounding the ovary; they then accumulate in the interstitial gland, around the germinal epithelial cyst and in the central connective tissue underneath this cyst, as well as around the medullary ducts, and they may invade also corpora lutea. However, they avoid the preserved follicles and only quite late and rather rarely do they invade the latter. In this case, the difference between their behavior towards different constituents of the same organ is almost as great as the difference in their reaction

would be against two distinct transplants, one autogenous and the other homoiogenous in nature. Two interpretations of this phenomenon are possible: (1) it may be assumed that different structures after transplantation produce and give off different quantities of the individuality differential; thus cartilage and fat tissue attract the lymphocytes in rat and guinea pig much less actively than does the thyroid gland. There is reason for assuming that the metabolically less active cartilage substance produces a smaller quantity of individuality differential per unit of time than does the metabolically more active thyroid gland. However, this interpretation probably does not apply to the ovarian structures; preserved follicles can hardly be less active in the production of individuality differentials than is interstitial gland tissue, or even corpus luteum tissue; (2) it is more likely that, in this and in other similar or even more striking cases, as for instance, in adrenal gland transplants in mouse, in addition to the individuality differentials certain tissue-specific substances attract the lymphocytes; but at the same time it is probable that, in this instance, also the individuality differentials play a role, their action being much re-inforced by that of substances given off by certain types of cells and tissues and not identical with the individuality differentials. It is conceivable that these two substances—the individuality differentials and the tissue-specific substances—are chemically linked to each other and that this combination exerts its influence on the lymphocytes, either directly or in conjunction with the individuality differentials circulating in the bodyfluids of the host. Such an interpretation seems more probable than the assumption that the interaction of the mutually not quite compatible individuality differentials of host and transplant interfere more effectively with the metabolism of some types of cells than with that of others, and that as the result of this interference, substances are produced which attract the lymphocytes. In the transplant of the adrenal gland of the mouse lymphocytes are attracted in large masses if certain regressive, degenerative changes have taken place in the cortical cells, provided this degenerative process does not exceed a certain limit. The rapidity and frequency with which this regressive stage is reached in the cortical cells is greatly influenced by the relationship between the individuality differentials of host and donor. In addition to the interpretations mentioned already, after all we cannot altogether exclude the possibility that in these cells larger amounts of individuality differentials are produced than in the well preserved cortical cells. Also to be considered is the possibility that in certain well preserved tissues influences inhibiting the invasion of the lymphocytes may exist. These are problems which still remain to be solved.

(b) *Syngenesiotransplantation in guinea pigs.* As in rats, so we compared in guinea pigs transplantations between brothers and sisters, from parents to children, and from children to parents. In addition, transplantations from grandparents to grandchildren and from grandchildren to grandparents were carried out. The guinea pigs were either very young, as yet sexually immature, or they were young adults; also, the rats used in the experiments already reported had been young.

In the guinea pigs we carried out three series of experiments; the largest

number of transplantations was made in the third series. The time of examination varied between 7 and 40 days in the different experiments; only those transplants examined not earlier than 17 days after transplantation were included in series III, while in series II some experiments are included in which the examination took place at an earlier time.

TABLE II

	Series I	Series II	Series III
Homoiogenous transplantations	1.85		
Brother to brother or sister	2.34	2.78	2
Parents to children	1.97	2.47	1.55 (1+/2−)
Children to parents	3 (only 1 experiment)	1.84	1.70 (2−)
Grandparents to grandchildren			1.75 (2−)
Grandchildren to grandparents			1.55 (1+/2−)

The average results obtained are shown in the accompanying table II. There is only one average figure for homoiotransplantation given; it was obtained in series I and it is probably too high; this is due very likely to the fact that only a rather small number of transplantations was made, and that among these there were two grades which exceeded the usual range in homoiotransplantation, one of them closely approximating the results obtained in autogenous transplantations. It is possible that in this instance we had to deal with related guinea pigs. If we make allowance for this discrepancy, the table shows that the grades in syngenesiotransplantations are higher than in homoiotransplantations, but that they are nearer those characteristic of homoiogenous than of autogenous transplantations. This was true also in the experiments with rats, which we have already discussed.

Taking these grades as a whole, the results of the syngenesiotransplantations are intermediate between those obtained in autogenous and homoiogenous transplants in either of two ways: (1) In a number of experiments the grades in the individual experiments vary, approaching either the results in autogenous or in homoiogenous transplantations. In these cases it is merely the averages which are intermediate. (2) In other transplantations the grades of the individual experiments are intermediate. In order to understand the manner in which such an intermediate condition may come about, we may distinguish in the reaction against the transplants on the part of the host cells, two periods, the first one tending from 6 to 12 days following transplantation, the second one covering the time from the 12th day to the time of examination. The reactions in the thyroid gland may be cited as an example of varying results in these two periods. During period I, the connective tissue reaction takes place. If during this time the production and accumulation of injurious individuality differential substances around and in the transplant has been strong, there is an active ingrowth of connective tissue cells towards the center of the graft and this tissue soon becomes transformed into hyaline substance; the ingrowth

of blood vessels is very limited. But if there is no or only a slight accumulation of injurious substances in this earlier period. then the connective tissue cells form a myxoid connective tissue along the inner margin of the ring of acinar tissue and there is here a good vascular supply. But in this case a lymphocytic reaction may be expected to set in sometime during period II, whenever toxic individuality differential substances produced have accumulated in sufficient quantity to attract lymphocytes in larger numbers; these latter then invade the graft, perhaps together with a restricted amount of connective tissue, which may move between and separate some of the acini. However, in general the severity of the reaction against the transplants increases with increasing time following the grafting and the earlier syngenesio-reactions may gradually become converted into severe homoio-reactions through intensification of the fibrous-tissue reaction together with lymphocytic infiltration, both of these factors leading to an increasing destruction of the transplanted thyroid. In cases in which paired organs from one donor were transplanted to two brothers, or from a child to both parents, the reaction in the two hosts was about the same in the majority of instances; this happened especially in those instances in which the reaction was severe. Under these conditions, a differentiation between results obtained in these hosts could not very well be expected. However, sometimes the reaction differed in the two hosts: there could be a severe lymphocytic reaction in the one and a slight reaction in the other, and such a separation of the reactions occurred also when the parents were derived from different strains of guinea pigs—one, for example, being curly and the other smooth-haired. It was noted in such a case that the reaction against tissues exchanged between parents and children was severe, while on the contrary, there was some evidence that, when the parents might have been related to each other, the reaction against the transplanted tissues was relatively slight. In those instances in which two organs such as thyroid and ovary were transplanted from the same donor into the same host, the reactions against both organs corresponded to each other.

In a general way, the grades were highest in the brother-to-brother (sister) transplantations, and there was no distinct difference between the two reciprocal types of transplantations when tissues were exchanged between parents and children. Such a result might be expected when random transplantations between non-inbred families of guinea pigs were carried out. In the rat, no definite difference between the three types of syngenesiotransplantation was noticeable.

Chapter 7

The Individuality Differentials of Closely Inbred Animals

CLOSELY INBRED animals are those which have been bred by brother and sister matings in a sufficiently large number of successive generations. As the result of this procedure, these animals have a genetic composition which has become even more similar than that of ordinary brothers and sisters; they exemplify an intensified brother and sister relationship. This close relationship should exist even between animals which do not belong to the same litter, but which have common ancestors in not far distant generations, and after very long-continued inbreeding, also between animals whose common ancestors are somewhat farther removed.

Theoretically, after from eight to ten consecutive brother-sister matings, the genetic composition of different individuals should be about the same (Sewall Wright); their individuality differentials should then be almost as nearly related as are those of different parts of the same organism or of identical twins. However, our transplantation experiments have shown that such an identity of individuality differentials among different members of the same closely inbred strain or family is approached with very much greater difficulty than would have been anticipated. As factors which might prevent or delay a homozygous state, we have, in the first place, to consider mutations in the germ cells, which may be expected to take place spontaneously and with a frequency which is not yet known. In the second place, a selection of the animals to be mated might influence the results. Thus Dr. Helen D. King, in her inbreeding experiments, selected in every case the most vigorous rats for breeding; this might imply a selection of the most heterozygous individuals, those which differ most in their genetic constitution and in which the individuality differentials are most dissimilar from those of brothers and sisters. Such a process of selection might delay the attainment of perfect homozygosity in the closely inbred strains, but this retardation would probably not be of very great consequence. A third factor involves the relationship between the animals in the first brother-sister mating; if these two individuals are very different in their genetic constitution, a greater number of consecutive generations of brother-sister matings will be required to produce homozygosity than if they are very similar to each other, and lastly there exists the possibility that a difference in the individuality differentials between host and donor of a transplant will be found if a branching-off from the common line of descent has taken place at a certain point and if the two individuals whose individuality differentials we wish to compare belong to different branches; the difference thus developed should be greater the further back the branching-off from the common line of descent occurred.

We carried out experiments (1) with rats closely inbred by Dr. Helen D. King at the Wistar Institute; (2) with guinea pigs closely inbred at the Department of Agriculture by Dr. Sewall Wright and Dr. Eaton, and later by Dr. McPhee and Dr. Eaton, and lastly, (3) with various strains of mice closely inbred by Mr. Marsh of the State Institute for Study of Malignant Diseases in Buffalo, and others closely inbred by C. C. Little and L. C. Strong and their associates at the Jackson Laboratory in Bar Harbor. A small series of experiments with closely inbred chickens, obtained from Dr. H. D. Goodale in Williamstown, have already been mentioned.

(a) *The individuality differentials in closely inbred rats.* We shall now discuss, first, investigations made with the closely inbred rats of Dr. Helen D. King, who had developed two distinct inbred strains, A and B; these had the same origin but they had been bred separately for many generations and thus had acquired in the end distinct genetic constitutions and individuality differentials; in addition, hybrids between strains A and B were obtained. Three series of experiments were carried out with these animals. In the first one, made mainly in 1926, rats from families A and B, belonging to generations 37

TABLE III
(Series I)

Donor and Host	Grades	Combined Grades
A to A (different litters)	1.82 (24 rats)	1.87 (51 rats)
B to B (different litters)	1.92 (27 rats)	
A to A (brothers and sisters)	1.68 (12 rats)	2.26 (36 rats)
B to B (brothers and sisters)	2.55 (24 rats)	
A to B	1.67 (18 rats)	1.62 (37 rats)
B to A	1.57 (19 rats)	

TABLE IV
(Series II)

Donor and Host	Grades	Combined Grades
A to A (different litters)	1.16 (16 rats)	1.49 (49 rats)
B to B (different litters)	1.65 (33 rats)	
A to A (brothers and sisters)	2.60 (17 rats)	2.71 (36 rats)
B to B (brothers and sisters)	2.81 (19 rats)	
A to B or B to A	1.37 (32 rats)	
Homoiotransplantation in non-inbred families	1.36	
(A×B)F_4 (or F_5) to (A×B)F_4 (different litters)	1.29 (12 rats)	
(A×B)F_4 (or F_5) to (A×B)F_4 (brothers and sisters)	1.80 (26 rats)	
A or B to (A×B)F_4	1.50 (13 rats)	
(A×B)F_4 to A or B	1.39 (13 rats)	

and 38, 40 and 41, and also 46 and 47 were used; in the second series made in 1930 and 1931, the rats belonged to the 60-67 generations, and in the third, most recent series, made from the year 1939 to 1941, a smaller number of rats

came from generations 91 and 92, and a larger number from generations 102 to 106. Somewhere between generations 92 and 102, family B died out and from then on only strain A and hybrids between strains A and B were still available. There was therefore a wide range of inbred generations, extending from the 36th to the 106th, and a time span of about 15 years in the progressive inbreeding of the rats, which were used in these experiments.

Series I (Table III). The grades in transplantations in different litters in inbred strains are slightly better than the average grades in homoiogenous transplantations in non-inbred rats and the grades are somewhat higher in strain B than in strain A. The combined grade of the transplants between brothers and sisters in strains A and B is higher than the grade of transplants between different litters. This is quite definite in strain B, while in strain A there is no marked difference between the two grades; the reaction happens to be even slightly less severe in transplants between rats belonging to different litters. Transplants from family A to family B (grade 1.67), and from family B to family A (grade 1.57) may serve as controls. Both these average grades correspond about to the grades of ordinary homoiotransplants; they are lower than the average grades of transplanted tissues exchanged within family A or family B. From these data we may conclude that as a result of close inbreeding in rats for 37 to 47 generations in families A and B, only a very slight progress towards a homozygous condition has been accomplished.

Series II (Table IV). A comparison of the grades in series I and II shows that there is an improvement in the grades in transplantations between different litters neither in family A nor in family B in series II over the corresponding grades in series I. However, in the second series, in both families the grades obtained in transplantations between litter mates are not only better than the grades obtained in transplantations between different litters, but they are also better than in the transplantations between litter mates in series I. Also, the grades in transplantations between hybrids $(A{\times}B)F_4$ or F_5 are improved to a certain degree if litter mates are used. But in these transplantations the results are not so good as in transplantations between litter mates in families A or B. Such a difference might be expected, because in hybrids there is a greater chance for unlike genes to accumulate in brothers when both A and B contribute to the genes of the fertilized germ cells. Transplantations from parent to hybrid give somewhat better results than the reciprocal transplantations, but both elicit severe homoiogenous reactions as an indication that a homozygous genetic constitution has not yet been reached in either family A or B. Exchange of tissues between families A and B likewise corresponds to a severe homoio-reaction, in accordance with expectations.

It is especially the results of transplantations between litter mates in inbred strains A and B which suggest that some progress towards a greater homogeneity in the constitution of the individuality differentials has been made through continued close inbreeding. In the exchange of tissues between brothers and sisters of the same inbred family, the factor of a difference in the distance of relationship between different litters is eliminated. It is not probable that the lack of a diminution in the severity of the reaction in the exchange of

tissues between different litters of the same inbred family is entirely due to a greater distance of relationship between these litters under these conditions, although this factor may play a certain role, but that in exchange of tissues between members of different litters the threshold required for a mitigated reaction had not yet been reached in series I as well as in series II. In the case of brothers and sisters it is probable that the decrease in the number and kind of unlike genes which were present in different individuals helped to make the individuality differentials so much alike that the reaction against the strange individuality differentials was diminished. This increased similarity in the constitution of the individuality differentials between brothers and sisters of these inbred strains, which in many cases approached an autogenous state, was brought out also in multiple simultaneous transplantations of various tissues into the same host; here all the tissues behaved like autogenous transplants, which is in accordance with the general rule that in transplantations from the same donor to the same host, all tissues behave in the same way if we make allowance for certain complicating factors, which we have discussed previously. Very instructive was also an experiment in which thyroids with adjoining tissues were successfully grafted into brothers and sisters. After two successive transplantations, and 50 days after the first transplantation, thyroid, parathyroid and fat tissue behaved like autotransplants; but after the third transplantation, the 73-day-old transplant showed a definite lymphocytic infiltration, although otherwise it behaved like an autotransplant.

This experiment confirms the conclusion that in series II a complete autogenous state has not yet been reached between brothers and sisters; but on the other hand, it is probable that a further progress, although a not very considerable one, towards a homozygous condition in families A and B has been made in continued propagation by brother-sister matings in the interval between the 37th to 47th generations and the 60th to 67th generations. However, in addition the strength of the reaction may depend not only on the genes of the donor, which are strange to the host, but also on the genes of the host, which differ from those in the donor, although the importance of the strange host genes is presumably less than of those of the donor.

Series III. In this series, as a rule, thyroid, cartilage and fat tissue, as well as pieces of striated muscle tissue, were transplanted and examination took place 20 days later. Two groups of experiments were carried out. In the first group (1) the hosts and donors were young rats, varying in age between about one and three months. In the second group (2) the age of the animals ranged between four and seven months. The results obtained in these experiments are shown in table V.

If we compare the transplantations in young rats (group (1)) and in somewhat older rats (group (2)), we notice that grafts between brothers and sisters, in family A in group (1), behave about like autotransplants, while in group (2), they behave like good syngenesiotransplants. The transplants between different litters of the inbred family have grades corresponding to those between good syngenesiotransplants; again, the grades are slightly better in group (1). A comparison with series I shows that transplants between differ-

ent litters as well as those between brothers and sisters elicit a much less antagonistic reaction in series III than in series I. As compared with series II, the grades are higher in series III in transplantations between different litters.

TABLE V
(Series III)
Group (1) Young rats. Group (2) Older rats.

Donor and Host	Grades	Grades
A to A (different litters)	2 82 (7 rats)	2.72 (10 rats)
A to A (brothers or sisters)	3.10 (22 rats)	2 77 (7 rats)
(A×B)F_4 and (B×A)F_4 (different combinations, different litters)	3.12 (6 rats)	2.18 (2 rats)
(A×B)F_4 to (A×B)F_4 or (B×A)F_4 to (B×A)F_4 brothers or sisters	3.12 (6 rats)	
A to (A×B)F_4		2.39 (4 rats)
(A×B)F_4 or (B×A)F_4 to A	2 87 (10 rats)	2.23 (4 rats)
B to A (91–92 generations)	1.48 (7 rats) 20 days 1.84 (7 rats) 12 days	

In transplantations between brothers and sisters the results are better in young rats of group (1) in series III than in series II, but about the same in the older rats of group (2). We may then conclude that considerable progress has been made in the direction towards a homozygous condition from series I to series III, and that progress has also been made from series II to series III, although even in series II the reactions between brothers and sisters were much less antagonistic than in series I.

In all three series the reactions against transplants between different families (A and B) were about alike and corresponded to homoiogenous relations of the individuality differentials. The grades, both in the transplants between different litters as well as between brothers and sisters of the hybrids (A×B), corresponded to autogenous relations of the individuality differentials in group 1. In group 2, the grades of transplants between different litters of hybrids were those of an average *syngenesio-reaction; they were much less favorable in* group 2 than in group I. But the reactions in groups I and 2 of series III were much better than the corresponding reactions in series II. Also, the reactions in transplantations from Family A to the hybrid or from the hybrid to Family A were much milder in series III than in series II. In series III these reactions in young rats of group 1 corresponded to good syngenesiotransplantations, while in the somewhat older rats of group 2 they corresponded to syngenesioreactions of medium intensity. The average was slightly, but not markedly, higher in transplants from family A to the hybrid than in the reciprocal transplantations. Similar results in this regard were obtained in series II, and this might be expected if a completely autogenous condition of the individuality differentials had not yet been attained in the families A and B.

Of interest in the third series is also the difference in the grades in the

tissues between different litters of the same inbred family is entirely due to a greater distance of relationship between these litters under these conditions, although this factor may play a certain role, but that in exchange of tissues between members of different litters the threshold required for a mitigated reaction had not yet been reached in series I as well as in series II. In the case of brothers and sisters it is probable that the decrease in the number and kind of unlike genes which were present in different individuals helped to make the individuality differentials so much alike that the reaction against the strange individuality differentials was diminished. This increased similarity in the constitution of the individuality differentials between brothers and sisters of these inbred strains, which in many cases approached an autogenous state, was brought out also in multiple simultaneous transplantations of various tissues into the same host; here all the tissues behaved like autogenous transplants, which is in accordance with the general rule that in transplantations from the same donor to the same host, all tissues behave in the same way if we make allowance for certain complicating factors, which we have discussed previously. Very instructive was also an experiment in which thyroids with adjoining tissues were successfully grafted into brothers and sisters. After two successive transplantations, and 50 days after the first transplantation, thyroid, parathyroid and fat tissue behaved like autotransplants; but after the third transplantation, the 73-day-old transplant showed a definite lymphocytic infiltration, although otherwise it behaved like an autotransplant.

This experiment confirms the conclusion that in series II a complete autogenous state has not yet been reached between brothers and sisters; but on the other hand, it is probable that a further progress, although a not very considerable one, towards a homozygous condition in families A and B has been made in continued propagation by brother-sister matings in the interval between the 37th to 47th generations and the 60th to 67th generations. However, in addition the strength of the reaction may depend not only on the genes of the donor, which are strange to the host, but also on the genes of the host, which differ from those in the donor, although the importance of the strange host genes is presumably less than of those of the donor.

Series III. In this series, as a rule, thyroid, cartilage and fat tissue, as well as pieces of striated muscle tissue, were transplanted and examination took place 20 days later. Two groups of experiments were carried out. In the first group (1) the hosts and donors were young rats, varying in age between about one and three months. In the second group (2) the age of the animals ranged between four and seven months. The results obtained in these experiments are shown in table V.

If we compare the transplantations in young rats (group (1)) and in somewhat older rats (group (2)), we notice that grafts between brothers and sisters, in family A in group (1), behave about like autotransplants, while in group (2), they behave like good syngenesiotransplants. The transplants between different litters of the inbred family have grades corresponding to those between good syngenesiotransplants; again, the grades are slightly better in group (1). A comparison with series I shows that transplants between differ-

Chapter 8

Individuality Differentials in Closely Inbred Guinea Pigs

THREE SERIES of experiments were carried out with guinea pigs. In the first series (1927), the guinea pigs were closely inbred in the Department of Agriculture in Washington, by Sewall Wright and O. N. Eaton. In a second supplementary series (1931), these experiments were continued with guinea pigs inbred for a few additional generations, likewise in the Department of Agriculture, by Hugh C. McPhee and O. N. Eaton; and a third, shorter and more recent series was carried out with guinea pigs closely inbred for only five or six generations at the Caworth Farms. The guinea pigs in the first series had been inbred by consecutive brother-sister matings mostly for from 17 to 23 generations, while the large majority of those in the second series had been inbred for from 20 to 25 generations. In the first series, five inbred families were used, designated as 2, 13, 32, 35 and 39; 2N was a line of family 2, selected for colored nose spots. This line was exceptional, insofar as it was not strictly propagated by brother-sister matings, but mating took place with a view of increasing the proportion of animals carrying this characteristic nose spot, without regard to relationship. It happened, however, that there were several brother-sister matings in this line, and in some instances they were repeated for three or four successive generations, according to information given me by Dr. O. N. Eaton. The degree of homogeneity in the various families differed (Sewall Wright, Bull., U. S. Dept. Agriculture, No. 1090). As controls, guinea pigs from a non-inbred B group and non-inbred guinea pigs obtained from various dealers were used. In the second series, only hybrids CY between families 2 and 13y, the latter an otocephalic line of family 13, and hybrids CP between families 13y and 35 were used as donors and hosts in the transplantation experiments. The figure following the designation of the family indicates the number of consecutive brother-sister matings. In the hybrids, the upper family represents the male and the lower the female partner. The figure following CP or CY signifies the number of generations of brother-sister inbreeding which the hybrids had undergone. CP-0 and CY-0 represent the F_1, CP-1 and CY-1 represent the F_2 generations, and so on. The guinea pigs in these experiments ranged in weight between 200 and 500 grams. The examinations usually took place between the 20th and 60th day, but in some experiments they were made as late as about 3½ and 5½ months following transplantation. We shall state the principal results obtained, without distinguishing between series I and II.

A. (1) *Transplantations in the same inbred family, host and donor not being nearly related.* In transplantations from 32-17 to 32-19 and in other similar transplantations in family 32 the grade was 3+. This indicates auto-

groups of the young and the somewhat older rats, which agrees with the general observation that when donors and hosts are very young, the reactions are milder than in older animals. This difference cannot be due to a lack of individuality differentials in the former, because such differentials are present; but it is due rather to a lesser sensitiveness to strange individuality differentials, or to a not yet fully developed mode of reaction in the younger animals. In addition, the fact must be taken into account that younger tissues grow more vigorously than older ones, and this condition is associated with a greater ability to overcome the effect of the antagonistic reactions of the host; it may also be that tissues growing more rapidly do not give off individuality differential substances in as large amounts as the more differentiated tissues in which the functional activity predominates. In accordance with these considerations, we noticed that in the group of younger rats the grades are higher, even in transplantations from hybrids to an inbred parent strain, where the derivatives of strange genes are introduced into the host.

A comparison of the reactions observed in these three series of transplantations shows that a continuous progress to a homozygous condition has been made. In the first series there was only a slight indication of an improvement in grades over the grades of ordinary homoiogenous and syngenesious transplantations. A further slight progress was noted in series II, but the greatest advance was made in the interval between series II and III. This means that after about forty generations, there was only a very slight progress towards an autogenous character of the individuality differentials; some advance was made after 60 to 67 consecutive brother-sister transplantations; but the greatest advance had been made when the 102nd generation was reached; however, even at that time no completely homozygous condition had as yet been attained. This finding is indicated especially by the transplantations into which the hybrids entered; but it is noticeable also in the transplantations within the inbred family A.

It seems most probable that the slow and imperfect progress in the direction towards a homozygous condition in the inbred rats is due to the occurrence of germinal mutations, leading to the introduction of strange genes into the constitution of host and donor and opposing, therefore, the attainment of an identity of the individuality differentials, which continued close inbreeding would otherwise more readily have accomplished. But of these two counteracting factors, germinal mutations and close inbreeding, the effects of the latter prove to be the more potent, and therefore the individuality differentials continue to progress on their way towards increasing mutual similarity, without, however, reaching this goal completely, even after as many as 106 consecutive brother-sister matings.

row, spleen, ovary, liver, adrenal gland, testicle and pancreas were transplanted, the grades were as follows: In two transplantations in family 32, and in one case in family 13, the grades were 3+, corresponding to autogenous transplantations. In three cases in family 13 and family 32, the grades were 3− and 3−/2+, corresponding to favorable syngenesiotransplantations, and in three experiments in family 2, the grades varied between 2+ and 2, corresponding to moderate or severe syngenesio-reactions. Liver and adrenal gland were not preserved at this late period, while they were well preserved after from 27 to 37 days; likewise, spleen was not well preserved. Pancreas was never recovered. Testicle tubules lined with Sertoli cells were seen. Again it was observed that the lymphocytic infiltration can occur at a late period, and that a fully homozygous condition has not yet been attained in the various families. In two additional experiments in family 2, the donors were the offspring of parents which represented hybrids between two different individuals belonging to family 2. For two generations this hybridization had taken the place of the usual brother and sister matings. The hosts were the offspring of continued brother-sister matings in family 2. The grades were 2 and 2.50, which correspond to average grades in syngenesiotransplantations.

The average grades in the various subdivisions of group A were as follows: 2.91; 2.87; 2.56; 2.99; 2.66. The total average grade was 2.72, which corresponds to a favorable syngenesio-reaction. This confirms the conclusion that a complete identity of the individuality differentials within the various closely inbred families of these guinea pigs has not yet been reached.

B. *Control experiments in which tissues were transplanted to non-related guinea pigs.* Examination took place 20 to 40 days later. (1) Transplantations from one inbred family to another inbred family: The grades varied between 2−/2 and 1. The average grade was 1.27, which corresponds to a severe homoiogenous reaction. The most severe reactions were obtained in transplantations in which family 13 was the host or was one of the constituents of a hybrid. (2) Transplantations from non-inbred B to B stock, or from hybrids between inbred families to B stock hosts: The average grade was 1.11. (3) Similar results, with an average grade of 1.11, were obtained in transplantations from B stock to St. Louis stock. In all these experiments the grades were characteristic of homoiogenous transplantations.

C. *Transplantations between brothers or sisters in inbred families.* Families 35, 32, 2 (2N) and 13 were used in these experiments, in which examination took place from 30 days to 5½ months after transplantation. In the transplantations in these various families, including family 13, the grade was 3+, except in one case, in 13-9, where it was 3−, corresponding to a favorable syngenesio-reaction. The somewhat lower grade in this instance was presumably due to the fact that here close inbreeding had not yet continued long enough. In two cases, transplantations between brothers which were hybrids of different generations within the same family were carried out, namely, in family 13 and in family 32; in both, the grade was 3+. As was to be expected, in the brother-to-brother transplantations autogenous reactions were approached to a still higher degree than in the transplantations between differ-

genous relationship between the individuality differentials of donor and host, but in another experiment in family 32, after 37 days the grade was 3/3−, corresponding to a very good syngenesio-reaction. In family 13, the grades varied between 3− and 3−/2+, even if both donor and host belonged to the 20th inbred generation. These grades correspond to good syngenesio-reactions, indicating the existence of some differences in the constitution of the individuality differentials. They may be due to the fact that, in family 13, there was a greater possibility that the number of direct common ancestors of different individuals was not so large as in the other families, and this may explain the lack of autogenous conditions, at least in some cases. In family 2, one grade was 3− (2-17 → 2-17), in another experiment it was 3−/2+ (2-18 → 2-16), an indication of a lack of identity of the individuality differentials. These results were, on the whole, confirmed by some experiments in which the examination took place at a later date. Thus, in family 13, after 5 months and 12 days, the grade was 2 (unfavorable syngenesio-reaction)—after 3½ months, it was 3. In family 2 (or 2N), autogenous conditions were found in these experiments.

These experiments indicate that in some families the autogenous condition was closely approached but was not yet quite attained, while in other families considerable progress had been made toward homozygosity, but the grades corresponded still to good syngenesio-reactions. In different experiments the results varied somewhat in various families. It is of special interest to note that the lymphocytic reaction may appear only at a late stage following transplantation, but that the lymphocytes may then exert a very destructive effect. Together with the late lymphocytic reaction there may occur a secondary slight proliferation of connective tissue in the transplant. However, the average grades in transplantations betwen guinea pigs belonging to different litters in these inbred families were higher than the average grades in transplantations between brothers or sisters in non-inbred families.

(2) *Successive transplantations of thyroid into the same inbred family.* These gave similar results. In family 32: First, transplantation for 37 days; second, transplantation to brother for 4 months, 9 days: grade 3−/3, which closely approaches results in autogenous transplantations. In family 13: First, transplantation for 31 days; second, transplantation for 32 days: grade 2, which corresponds to a severe syngenesiotransplantation; but in this case, donor and last host had only seven generations of brother-sister matings in common.

(3) *Multiple simultaneous transplantations.* In four experiments with families 13 and 32, several pieces either of thyroid gland alone, or of thyroid and various other organs (spleen, liver, adrenal, pancreas) were transplanted simultaneously: examination took place between 27 and 37 days. The grades varied between 3/3− and 3+, therefore between the reactions in favorable syngenesious and in autogenous transplantations. The results here were as good in family 13 as in family 32. In nine additional experiments with families 32, 2 and 13, in which the examination took place 4 months, 9 or 12 days after transplantation, and in which thyroid, cartilage-fat tissue, bone and bone mar-

13 as a host and to an increased injuriousness of its individuality differentials. In family 32, the opposite conditions obtain.

F. *Transplantations from a parent family to a hybrid.* Also in this group of experiments the actual parents of the hybrids were not used in these transplantations, but other members of the corresponding families. 16 experiments were carried out. Examination took place between 25 and 35 days. The reactions were much milder in this series than in the reciprocal series E, except in one case, in which 2N-52 was transplanted to a hybrid between families 2 and 13y (CY-0$\left\{\begin{matrix}\text{2-22}\\\text{13y-26}\end{matrix}\right.$. Here, the grade was 1. Whether in this instance the donor 2N happened to carry some genes strange to the family 2 component of the host, or whether some error entered into this transplantation, is uncertain. The fact may be recalled in this connection that the line 2N had not been propagated by strict brother-sister matings. Omitting this last transplantation, the average grade in the remaining 15 transplantations was 3.05, a grade similar to those obtained in transplantations between brother hybrids in inbred families, which were 2.94 and 3.12. If we include the transplantation with grade 1, the average grade was 2.92. If we omit again this one transplantation, there were six of 15 experiments in which the grades were below 3, namely, 2.87 and 2.75; these grades were characteristic of a favorable syngenesio-reaction, while in the other cases the reaction approached closely the autogenous state. Taken as a whole, the grades were very good in this series and they came near to those characteristic of autogenous reactions, but a fully homozygous condition had not yet been reached.

From these data, it may be concluded that transplantations from hybrids to one of the parent families did not elicit homoiogenous reactions, but, instead, severe syngenesio-reactions. It seems possible that in this case the presence of gene sets common to donor and host mitigated the intensity of the reaction of the parent-family serving as host against the strange component of the hybrid-transplant. On the other hand, transplantation from a parent-family to the hybrid-host did not give a perfect autogenous reaction, since some of the genes of the parent may be strange to the corresponding constituent of the hybrid; therefore, in a number of cases, syngenesio-reactions were obtained. Another possible factor affecting the result in these experiments was the apparent tendency of certain families to elicit a more severe reaction, or to respond more aggressively themselves than others; thus the involvement of families 13 and 35 in the transplantation seemed to cause a relatively severe, and that of family 32 a more mitigated reaction. As stated previously, there is some indication that the reaction may be stronger in the presence of family 13, because in this family the various members may have a smaller number of common brother-sister matings than in some other families; but such a condition should not affect the severity of the reaction of a family other than 13 serving as host against a hybrid containing the gene set of family 13; in this case, other conditions must be responsible for the severe effect.

G. *Transplantations between hybrids, in which donor and host were composed of the same two inbred families but were not brothers or sisters.* 14

ent litters of inbred families. In 10 transplantations, brothers or sisters which were hybrids between two individuals from families 32, 39, 35, 13 or 2 were used. In six of these transplantations one of the parents belonged to family 13, while the other parent belonged to families 32, 35 or 2. The grades were 3−, 3−, 3+, 3, 3, 3/3−. In the remaining four transplantations, the parents that entered into the hybrid composition belonged to families 39, 2, 32, or 35; the grades were 3, 3+, 3+, 3. In these two sets of transplantations, the average grades were 2.94 and 3.12, respectively. While the difference between these two averages is perhaps too small to be of much significance, both these grades are slightly less favorable than those obtained in brother-to-brother transplantations in the closely inbred strains. This is presumably due to the fact that in hybrids a summation of the effects of strange genes takes place, which latter may still be found in some individuals in inbred strains.

D. As controls, transplantations were carried out between brothers in non-inbred stock. In former experiments of this kind, the average grade was 2/2− (1.87). In 15 additional experiments in stock B guinea pigs, the average grade was 1.91. These grades are better than those obtained in transplantations between non-related, non-inbred guinea pigs, in which the average grades were 1.27 and 1.11, but they are much lower than the average grades obtained in transplantations between brothers in inbred strains.

E. *Transplantation from a hybrid between two inbred families to one of the parent families.* 25 experiments were made; in each case an F_1 hybrid was used as the donor. In no case was one of the actual parents used as host, but merely the families to which the parents belonged. Examination took place between 21 and 37 days after transplantation. The average grade was 1.75, which corresponds to a severe syngenesio-reaction, but is somewhat higher than the average grade found in homoiogenous transplantations. Here, again, it appeared that the severity of the reaction was strongest in transplantations in which family 13 was a component part of the donor, even if the host belonged not to family 13, but to the other parent family which entered into the hybrid. There were six transplantations in which family 13 entered as a parent strain in the donor; in three of these, strain 13 was likewise the host. The other parent family was 2. The grades in these six transplantations were as follows: 1.25, 1, 1.12, 1.25, 1, 1.25. The average grade was 1.14, which corresponds to a severe homoiogenous reaction. There were 13 transplantations in which family 32 entered into the hybrids, and in six of these it was also host. The grades were as follows: 2.12, 2, 1.87, 1.87, 2, 2, 3.25, 1.87, 3, 1.12, 3, 1.87, 2.75, corresponding to an average grade of 1.98. The average grade of the experiments in which strain 32 was also host was 2.5. The other component families were 2, 35 and 39. There remain six transplantations in which families 2, 35 and 39 entered; here the average grade was 1.65. There are, then, at least strong indications that the presence of family 13 in the hybrid intensified the reaction against the graft and that the presence of family 32 in the donor, and especially also in the host, mitigated the severity of the reaction. It is not probable that the strong reaction against family 13 is due actually to a lesser effect of the long-continued inbreeding, but to a greater reactivity of family

In a few experiments of the first series, in which brother-to-brother transplantations were carried out, the grade was slightly above 1; likewise, in transplantations from one strain to another the average grade was 1. In a second series of transplantations, made within the same strains, the average grade was 1.85 in six experiments. In six other experiments, in which transplantations were made between different strains from Caworth Farms guinea pigs to St. Louis guinea pigs, the average grade was 1.33. We may then conclude that there is perhaps a slight indication of a beginning homozygous condition in these guinea pigs, as indicated by grade 1.85 in transplantations within the same strain; but in the first series, there is no indication of such a tendency. Therefore, after five or six generations of inbreeding, there is not yet any definite advance in the direction towards a homozygous state among these animals.

The following are the principal conclusions suggested by all these experiments with closely inbred guinea pigs. The difference between the relatively rapid effect of inbreeding on the individuality differentials in guinea pigs as compared to the effect in rats, which were studied in the preceding pages, is striking. However, even in guinea pigs the ultimate goal of the inbreeding, namely, a completely autogenous state of the individuality differentials, of all the animals in an inbred family, has not yet been reached. The individuality differentials of host and transplant were the more similar to one another the larger the number of brother and sister matings which these individuals had in common before the separation of these matings into different sidelines took place, and the smaller the number of these separate and distinct brother-sister matings had been in the preceding generation in host and donor. The separation into side lines may cause an accumulation of unlike genes if a perfect homozygous condition had not yet existed at the time of the separation; mutations may then add to the number of unlike genes. In family 2, for instance, the grade was 2.50 in a case in which there had been six to nine common ancestral matings, followed by 20 to 24 separate brother-sister matings, while the grade was 3.25 with 12 and 16 brother-sister matings in common and subsequent separation for only two or three generations. Similar results were obtained in family 13. After 19 to 20 consecutive brother-sister matings, there was usually an absence of any marked incompatibility between the individuality differentials. Such individuals may behave like identical twins, at least within the range of conditions which were used in these tests; however, a lengthening of the time during which the individuality differentials of host and transplant had a chance to act on each other might still have brought out a certain difference when a shorter period did not show it, and some of our experiences indicate such an effect. Striking also is the correspondence between the pedigree relationship of the various guinea pigs and the degree of relationship of their individuality differentials as revealed by transplantation; this comes out especially in the brother-to-brother transplantations and in the various types of hybrid transplantations. It is interesting in this connection that in the experiments with inbred guinea pigs, as well as in those with inbred rats, the greater similarity of the individuality

transplantations of this kind were carried out; but in one of these, the examination took place as early as 10 days after transplantation. Omitting this case, there remain 13 experiments in which the examination took place between 20 and 40 days. In the large majority of these transplantations the hybrids were composed of families 13y and 2, but in a few instances, families 35 and 32 were parents of the hybrids. We may arrange these transplantations according to the similarity or difference in the generations of brother-sister matings of the two component parents in donor and host. In one set the parent families, 13y and 2, which entered into the hybrids in donor and host, belonged to the same generations: (Cy-0 $\begin{cases}13y\text{-}24\\2\text{-}23\end{cases} \rightarrow$ Cy-0 $\begin{cases}13y\text{-}24\\2\text{-}23\end{cases}$ (21 days); grade 3. In four transplantations, the generations were the same in one inbred family but different in the other family: CP-0 $\begin{cases}35\text{-}25\\13y\text{-}24\end{cases} \rightarrow$ CP-0 $\begin{cases}13y\text{-}25\\35\text{-}25\end{cases}$ (20 days); grade 2.75. The grades in the other three transplantations of this kind were: 3, 3.25, 2.75; the average grade was 2.94. In the remaining eight experiments, in each case the parents composing the hybrids belonged to different generations. The grades were: 1.75, 1.75, 1.75, 1.25, 1.75, 1 (hybrids between families 13y and 2), 2.75 (hybrids between families 2 and 35), and 3.25 (hybrids between families 32 and 35). Again the presence of family 13 in the hybrids seemed to intensify the severity of the reaction, while in the combination between families 32 and 35, the result corresponded to that found after autogenous transplantation. The average grade in the six transplantations into which family 13y entered was 1.54, which approached that of a homoiogenous transplantation.

H. *Transplantation in hybrids in which one of the two parent families was the same in donor and host, while the other parent family was different.* One hybrid was composed of families 2 and 13y, while the other hybrid, either host or donor, was composed of families 13y and 35. Three transplantations were carried out and examination took place after 19 to 21 days. The grades were: 1.87, 2.12, 1, and the average grade was 1.69, which represents a moderate homoiogenous reaction. The result is similar to that obtained in the transplantations between hybrids 2 and 13y, in which both parent families belonged to different generations.

Series III. Transplantations between guinea pigs during early stages in inbreeding.

Two short series of experiments were carried out with guinea pigs which had been inbred for five to six generations by brother-sister matings at the Caworth Farms in New York. We shall briefly mention these transplantations, because they indicate that in contrast to the experiments with guinea pigs which had been subjected to close inbreeding for from about 15 to 27 generations, no definite progress towards an autogenous condition of the individuality differentials had as yet been made. Guinea pigs from two strains (CP and Connaught), in each of which there were several inbred families, were used. The initial weights varied between 225 and 310 grams and examination took place after 30 days. Thyroid, cartilage, fat tissue and muscle or thymus were transplanted.

in the intensity of reactions of hosts against transplants. Although, as stated, it is much easier to bring about a great similarity in the constitution of the individuality differentials through continued close inbreeding of certain families in guinea pigs than in rats, we have seen that even in guinea pigs, after a considerable number of generations of brother-sister matings, a complete identity of the individuality differentials has not yet been reached. In accordance with this fact is the observation that, a small number of generations of brother-sister matings does not seem to cause a marked increase in the similarity of the individuality differentials in guinea pigs, as shown in series III of these experiments.

differentials in brothers and sisters, as compared to non-litter mates, was very definite, in contrast to experiments in non-inbred animals, where these differences between syngenesio- and homoiotransplantations are not always definite. The correspondence between these two variables, pedigrees and individuality differentials is, on the whole, very close, and this is one reason why we thought it worth while to give some of the results obtained in these transplantations with greater detail. Of further interest is the great difference between the results of hybrid-to-parent and the reciprocal transplantations. The former are not, however, identical with homoiogenous transplantations, nor the latter with autogenous ones; but in both intermediate results were obtained, suggesting that the presence in the hybrid of a gene set similar to that in the host may have beneficial effects, while the presence of unlike factors in the host may intensify unfavorable reactions against the transplant.

It is conceivable that the presence in the host of a set of genes not present in the donor might lead to differences between the metabolism of host and transplant and that this might, to some degree, influence the aggressive action of the host against the transplant. However, the intensity of the reaction in these transplantations depends essentially upon the presence in the donor of genetic factors not present in the host. In tumor transplantations, Little and Tyzzer (*1916*) found that in transplantations from hybrids to parent strain, no successful transplantations were obtained, while the reciprocal transplantations gave 100 per cent takes; they concluded that only one dose of genes is required for successful transplantations. Subsequently (1921/22), Little and Johnson noted that if pieces of spleen were transplanted from inbred Japanese mice to hybrids between the Japanese and white mice, the results corresponded to those of autotransplantation, while in reciprocal transplantations from hybrids to waltzing mice, the transplants were destroyed in a short time, indicating a strong homoio-reaction. Similar results were published more recently by Little and Bittner. But in these experiments, conditions were selected which made the recognition of intermediate results very difficult. This caused a lack of the finer means of measuring existing differences between the individuality differentials of donor and host, and led to the assumption of a genetic identity in one type of transplantation, and of a complete lack of similarity of the individuality differentials of host and donor in the reciprocal type, whereas, in all probability, various kinds of intermediate states existed.

And, lastly, there are at least indications that there exist constitutional inherited differences between some of the inbred families, which determine not only differences in the intensity of their reactions against strange individuality differentials in the transplant, but which also may perhaps influence the degree of toxic action which the transplant exerts on various hosts. While differences in the interaction between hosts and transplants in different experiments may be partly explainable on the basis of differences in the number of generations which have passed since the ancestries of two individuals, belonging to the same family, branched off from each other, it is not probable that this is the only factor determining the differences observed

direct parents to the hybrids, although it still falls into the range of nearly related individuality differentials. Decidedly more unfavorable were transplantations from hybrids $(C57xD)F_1$ to their own parents, with an average grade of 1.42 in ten transplantations, or from such hybrids to members of their parent strains, other than their actual parents, with an average grade of 1.50 in five transplantations. Both of these reactions fall into the range of homoiogenous relationship of the individuality differentials. In all these experiments examination as a rule took place 20 days following transplantation, and each mouse received transplants of thyroid, cartilage, fat tissue and striated muscle; whenever possible, also ovaries were transplanted. These grades indicate very strongly that neither in strains C57, D, New Buffalo, nor even in strain A, has an autogenous condition of the individuality differentials been reached; but it has progressed farthest in this direction in strain A. All these grades fall into the range of syngenesiotransplantation. However, the reactions are much milder in intrastrain than in inter-strain transplantations, where they correspond to homoiogenous transplantations. In accordance with expectation, the reactions were more severe if instead of transplanting within the same inbred strain, we transplanted between hybrids such as $DxC57)F_1$, in which both parents were from inbred strains. In this case the results were intermediate between those obtained in simple inter-strain and intrastrain transplantations, corresponding to unfavorable types of syngenesiotransplantation. However, if the hybrids which served as donors and hosts were brothers and sisters, then the results approached more closely those obtained in autogenous transplantations. As was to be anticipated, in transplantations from parent strains to hybrids the grades were higher than in the reciprocal transplantations. In the former, they corresponded about to the grades of simple intra-strain transplantations, although they were slightly better than the latter if the actual parents of the hybrids served as donors; however, an autogenous condition had not yet been reached. The reciprocal transplantations from hybrids to parent strains or to the actual parents gave results which were only slightly better than those obtained in inter-strain transplantations.

As stated on previous occasions, in the mouse the main reliance in the grading must be placed on the state of preservation of the various tissues, on the kind of structures which survive, and on the extent of the ingrowth of connective tissue into the various transplants and the degree of replacement of the latter by fibrous tissue. In the case of the fat tissue, also the number of epithelioid phagocytic cells which separate the fat cells is significant. While lymphocytic infiltration plays a certain role likewise in the mouse, and while it may be very intense in some instances, it can not be relied upon as a quantitative standard in the evaluation of the reactions to the same extent as in rat and guinea pig; but in the mouse, also, there is a very good correspondence beween the fate of various kinds of tissues transplanted from the same donor into the same host, if we make allowance for peculiarities of different types of tissues and for accidental variable factors which may complicate the results.

Chapter 9

Individuality Differentials in Closely Inbred Strains of Mice

We have already discussed the transplantations of tissues from one closely inbred strain of mice to another, as well as some transplantations in non-inbred mice, and we shall later analyze also heterogenous transplantations between mice and some other species of animals. We shall now consider the transplantations within inbred strains—intra-strain transplantations—the character of the individuality differentials of mice belonging to such strains, and the effects of hybridization between different inbred strains on the character of the individuality differentials of the hybrids.

From among the large number of these types of transplantation carried out, we shall first select a smaller representative group, in which, to a large extent, some of the complicating factors, as indicated by the presence of polymorphonuclear leucocytes in the transplants, have been avoided. We shall then analyze the results of other groups of intra-strain transplantations, as well as transplantations between hybrids, and some experiments in which the analysis of the complicating factors mentioned, as well as of some additional ones, has been attempted.

In inter-strain transplantations between strains C57 and D, which we have already discussed, the average grade of ten transplantations was 1.29. This corresponds to a homoiogenous relationship of the individuality differentials. In ten transplantations between *not* closely related mice belonging to the same inbred strains C57 or D, the average grade was 2.26, which corresponds to syngenesiotransplantation. The grades varied between 2 and 3.12, but in one case the grade was as low as 1.75. In four transplantations within inbred strain A, the average grade was somewhat higher, namely, 2.81. In two New Buffalo mice the average grade was 2. These grades varied between those characteristic of favorable and of average syngenesiotransplantations. In eight transplantations between hybrids $(DxC57)F_1$, belonging to different litters, the average grade was 1.91, which, in accordance with expectation, is below the grades obtained in grafts between members of inbred strains. In 11 transplantations between hybrids $(DxC57)F_1$, which were brothers, the average grade was much higher, namely 3.04, a result which approaches that found in autogenous conditions. In 21 transplantations from parents D or C57 to their hybrid children $(C57xD)F_1$, the average grade was 2.60, which corresponds to a favorable syngenesio-reaction. In case members of the strains to which the parents belonged, but not the parents themselves were the donors, the average grade in six transplantations was 2.35, which is slightly less favorable than the transplantations from the

tissue and lymphocytes, a destruction of a part of the thyroid transplant, and an infiltration of the fat tissue by connective tissue, lymphocytes and small vacuolated phagocytic cells. However, the more severe reactions may have been due to infection, as indicated by the presence of polymorphonuclear leucocytes in or around the transplants.

In *strain C57* we find, again, reactions varying between those seen in autogenous and those in homoiogenous transplantation. Besides the many experiments in which the results resembled or approached those obtained in autogenous transplantations, there were a considerable number of syngenesio- and homoiogenous reactions and, as a rule, a correspondence existed between the behavior of different transplanted tissues. In some instances, the presence of polymorphonuclear leucocytes made the interpretation of the results difficult.

In *New Buffalo strain*, in which donors and hosts were from 2 to 6 months old, either almost autogenous reactions or syngenesio-reactions were obtained; in some instances, a stunting of transplanted thyroids and striated muscle tissue was noted. The average grades were 2.84 in transplantations between litter mates and 2.81 in those between non-litter mates, a difference which is of no significance. In a few older mice belonging to *strain Old Buffalo*, a few homoiogenous reactions were noted. There was, as a rule, a correspondence in the type of reactions against different tissues in transplantations from the same donor to the same host. On the whole, then, the individuality differentials in strain New Buffalo approached identity, but this goal had not yet been quite reached.

In *strain AKA*, there was usually a definite homoiogenous reaction against thyroid as well as against cartilage and fat tissue. In some cases the thyroid was destroyed, in others there was an incomplete ring of acini invaded by fibrous tissue and with much lymphocytic infiltration, which helped to destroy the acini. In such cases the fat tissue was usually invaded by fibrous tissue, lymphocytes and phagocytic, vacuolated cells; the bone marrow was necrotic. In one instance, the thyroid transplant approached an autogenous condition, and, correspondingly, there was very little connective tissue ingrowth or lymphocytic infiltration in the fat tissue. In a few other cases there were syngenesio-reactions; but for the most part, a marked homoiogenous reaction was found.

In *strain C* only a few transplantations were carried out; the results were good, approaching autogenous or syngenesious conditions.

Do variations in age of donors or hosts in inbred strains affect the reactions against the individuality differentials? In further experiments we tested the degree to which the individuality differentials, within the various inbred strains, had become similar to one another or identical, by transplanting tissues from relatively old donors to young hosts and vice versa. This procedure gave us also an opportunity to study further the effect of the age of donors and hosts on the reactions against individuality differentials, in experiments which can be more readily carried out in mice than in other species, because mice undergo old age changes and die earlier than do most other mammalian species used in these experiments.

A comparison of the results of transplantations within various inbred strains. In a further extensive series of experiments we have compared, in a number of closely inbred strains, the reactions against transplants, when donors and hosts belonged to the same inbred strain. (1) In *strain A*, pieces were examined from 12 to 50 days after transplantation; the age of the hosts and donors varied, as a rule, between 2 months and 7 months, but in some transplantations the hosts were as old as 16 months. These differences in age did not affect noticeably the results of transplantation. In the large majority of cases the transplants (thyroid, parathyroid, cartilage, fat tissue, bone and bone marrow, and striated muscle tissue) behaved like autogenous transplants, or they at least approached this condition. But in certain instances the thyroid graft was stunted or there were some mild lymphocytic infiltrations in the thyroid, muscle or cartilage-fat transplants. These changes were found as early as 20 days after transplantation and also after 50 days; but when present, they were mild and they corresponded to favorable syngenesio-reactions. There was a marked similarity in the condition of the various tissues transplanted from the same donor into the same host—they all showed the structure of autogenous transplants—although, as stated, there could develop a mild lymphocytie infiltration in some instances. More severe reactions were noted in experiments in which the presence of polymorphonuclear leucocytes, which accumulated especially inside of fibrous nodules, indicated a probable infection. At some distance from the center of infection an increase in lymphocytes in the fat tissue and infiltrations with small vacuolated phagocytic cells could be noted; but even under such conditions the injurious changes, including formation of fibrous tissue, as a rule were mostly localized and did not lead to a general damage of the transplants. In the majority of these, as well as of the following transplantations, examination took place after 20 or 30 days, but in some cases it was as late as 50 days, as it was also in other strains than A.

In *stroin C3H*, the results were similar to those found in strain A; in the majority of cases the pieces behaved like autogenous transplants; however, there were occasionally some slight lymphocytic infiltrations, and if an infection had occurred, the reactions were more severe.

In *stroin D*, a great variability of the reactions was noted; these ranged from an autogenous to a severe homoiogenous type, in which a great part of the thyroid was destroyed and the remaining acini were embedded in fibrous tissue. There was also some lymphocytic infiltration; in the fat tissue an increase in fibrous tissue had taken place, and epithelioid, small vacuolated phagocytic cells, together with some accumulations of lymphocytes, were present. In general, there was a correspóndence between the behavior of different tissues taken from one donor and transplanted into the same host. In an experiment in which four pieces of thyroid had been transplanted from two donors into the same host, two of the transplants behaved like autogenous, and the other two like homoiogenous transplants. Strain D is then, it seems, less homozygous than strains A and C3H.

In *strain CBA* the results corresponded mostly to those found in autogenous transplantations; but in some instances there was an increase in fibrous

transplantations between 2 months old mice, the results approached those found in autogenous reactions. These experiments confirm, then, our previous findings, which indicated great variations in the transplantations in this strain and the occurrence of autogenous as well as of homoiogenous reactions. We noted furthermore that transplants into hosts as old as 18 months may remain very well preserved, but in general the results were better in experiments in which host and donor were young than in those in which they were older.

In *strain C57*, almost all mice older than 12 months are affected to a variable extent with sclerosis of the thyroid gland. If the various organ pieces, including thyroid gland, of mice ranging in age between 15½ and 18 months, were transplanted into young, 2 to 3 months old mice, the preservation of the grafts was, on the whole, very good; but in one-half of the transplanted thyroids sclerosis was found, and in five out of six of these pieces there was much or moderate lymphocytic infiltration. Therefore, the sclerosis of the thyroid, which develops in older mice of this strain, may remain unchanged in young hosts.

In *strain C3H*, tissues were transplanted from 13 to 13½ months old mice to 3½ to 4 months old animals. The results approached those of autogenous transplantations and only rarely a slight lymphocytic infiltration was found. As controls, transplantations were made from strain C3H to strain C57. The donors ranged in age from 15 months and 13 days to 13 months and 20 days; the hosts' age ranged from almost 3 to 4 months. The average grade was 1.37, which corresponds to a marked homoiogenous reaction. In one instance the thyroid was partly sclerosed. The relatively old age of the donor did not modify noticeably the strength of the reaction of the host against the transplants, and the marked difference between the severity of the reaction after transplantation of tissues into different strains and into the same strain is quite evident.

In one experiment, four thyroid glands were transplanted from two 1½ months old CBA mice to a 19 months old CBA mouse. Two months later, at autopsy, two thyroids were found. There was no lymphocytic infiltration and the thyroid tissue was well preserved, but a small amount of hyaline tissue had developed around certain acini. Also, in the parathyroid hyaline septa were noted. This observation agrees with that made in some other instances in which thyroid glands were transplanted from young into old mice. We are inclined to interpret this condition as a partial sclerosis of the thyroid gland. It seems, then, that after transplantation of the thyroid gland from young into old mice of the same strain there may be, in some cases, relatively much development of dense fibrous-hyaline connective tissue around certain acini. The conditions usually present in the old hosts would favor sclerotic changes in the thyroid tissue, and if this tendency is sufficiently strong, it may lead to sclerosis even in the non-transplanted thyroid gland of the host; otherwise, there was no indication of a specific reaction of the host against the individuality differential of the transplant, and in some transplantations into old mice the results may approach those found in autogenous grafts.

These experiments confirm, therefore, our former ones, in which it has been

Strain A. In experiments in which the donors were from 14 to 19 months old and the hosts were young, the transplanted thyroid was found more or less sclerosed, which means that the individual acini were surrounded and separated from one another by hyaline-fibrous tissue. However, in strain A, beginning soon after the age of 12 months, the thyroid normally undergoes sclerosis, although not so frequently as in strain C57. The parathyroid showed corresponding pericapillary hyalinization. It follows that these local tissue changes cannot be reversed by transplantation into young hosts, where the constitution of the bodyfluids differs in certain respects from that in older animals. While in certain cases some of these transplants behaved like autogenous tissues, in some others the transplants were invaded by lymphocytes, slightly or even more extensively. The lymphocytic infiltration became greater, especially around the grafts which had been kept in the host for longer periods. This observation seems to confirm the conclusion that even in strain A the individuality differentials of different animals have not yet become identical. But, as we have found in other cases, here also local factors may co-operate with the effects of a certain degree of incompatibility between the individuality differentials in calling forth lymphocytic infiltration; thus we noticed in one instance in which two thyroids from the same donor were transplanted into the same host, one showed some lymphocytic infiltration while the other one was as yet free from it. Such a result may have meant merely that in one graft the threshold of stimulation for the host lymphocytes was reached somewhat earlier than in the other one. In reciprocal experiments, tissues (thyroid, cartilage and fat tissue, striated muscle) were transplanted from 2 or 2½ months old mice to 18 or 19½ months old hosts. Here, all the tissues were well preserved; the transplants behaved on the whole like autogenous grafts; it is possible that the amount of preserved thyroid or muscle tissue was somewhat reduced, although this does not seem to be a necessary result of the old age of the hosts. There was no sclerosis and no lymphocytic infiltration in the old hosts.

We compared with these intra-strain transplantations, inter-strain transplantations of the same kinds of tissues, in which both host and donor were about 4 to 5 months old. Here the reactions were much more severe. In some cases the thyroid transplant had been entirely replaced by fibrous tissue and lymphocytes, in others, more or less tissue had been destroyed. The average grades in these inter-strain transplantations were: Strain A to strain New Buffalo—1.06; strain New Buffalo to strain A—1.90.

In *strain D,* in transplantations from young 1½ to 2½ months old mice, to mice ranging in age between 12 and 19 months, the grades varied between 3, approaching grades given in autogenous transplantations, and 1+; usually there was a partial fibrosis, due to increase of connective tissue between acini and imperfect preservation of the transplants. In transplantations from 12 months old donors to 2 months old hosts the results were good, the grades being mostly 3 and 3—. In transplantations in which hosts and donors were about 7 months old, the results were intermediate, lymphocytes invaded thyroid and muscle and there were vacuolated phagocytic cells in the fat tissue. In

age of 13 months, and two the age of 12 months at the time the experiment was started. The serial transplantations were continued for periods that varied between 5½ months and 34½ months. The youngest thyroid recovered after completion of the serial transplantation was 11 months and 10 days old and the oldest one was 41½ months old; the age of the others was intermediate between these ages. The age of 41½ months exceeds considerably the average age of A mice and exceeds, probably, also the oldest age which mice belonging to this strain reach. The number of serial transplantations made in these various experiments ranged between three and seven, and the time during which a transplant remained in a single host varied between 2½ months and 6 months; but in several instances the transplant was left only 2½ weeks in the last host. On microscopic examination it was found that the thyroid was preserved, but in four experiments it showed either slight or partial sclerosis, while in six experiments it showed complete sclerosis. In the latter case, all the acini were surrounded by rings of hyaline-fibrous tissue, which separated the acini from one another. In the center, a number of acini had been reduced to thin cell strands or had been lost entirely through pressure of the stroma, which injured the epithelium, and, in addition, interfered with its nourishment. Several capillaries were seen, however, in the stroma. These thyroid transplants, therefore, resembled closely the non-transplanted thyroids of older mice belonging to strain C57. In four cases a limited number of acini were surrounded by thin hyaline rings; but over larger areas the acini were lying close together. Lymphocytic infiltration was either lacking entirely, or it was slight. Probably two factors are involved in the development of this sclerotic condition: (1) The age of the donor. An age of the donor above 13 months favors complete sclerosis. There was no sclerosis when the transplant was less than 12 months old. (2) The length of time during which the transplant remained in the strange hosts. Complete sclerosis was found in cases in which the donor was only 6 or 12 months old; here there is little doubt that the thyroid at the time of transplantation was not yet sclerotic, but that it acquired the sclerosis in the course of the serial transplantations. Therefore, if the transplanted thyroid remained long enough in different hosts, or if it attained a certain age, it tended to become completely sclerotic. This condition was especially marked if the donor of the thyroid had reached the age of 14 months, in which event the transplant either was already somewhat sclerotic or had a greater tendency to become so. This interpretation agrees with our findings in the preceding experiments, where there were likewise indications that both the factors mentioned here may play a role in producing thyroid sclerosis.

It follows from these experiments that by means of serial transplantations in the same inbred strains, it will in all probability be possible to keep whole organs alive up to an age which much exceeds that usually attained under normal conditions, and that the reactions of the host against such transplants may be lacking entirely. It may perhaps be possible even to keep such transplants alive indefinitely by serial transplantation. However, it may be suggested that in future experiments of this kind, the transplant be allowed to remain for longer periods of time in the same bost and that, correspondingly, the number

shown that there are still disharmonies between the individuality differentials of mice within the same inbred strains, but that the frequency with which the resulting antagonism is found varies in different strains. They show also that, essentially, old hosts react against transplants in a similar manner to young hosts, except that there may possibly be a greater tendency in the old hosts to the development of fibrosis around the transplanted thyroid acini; but in experiments in strain A, similar results were obtained in transplantations in which the age of donors and hosts, brothers and sisters in these cases, was 1 or 2 months and in others in which it was 11 months. In addition, these experiments show that if a sclerosed thyroid is transplanted to a young mouse belonging to the same strain, the sclerosis may persist and that such a sclerosed thyroid may also be invaded by lymphocytes.

Serial transplantation of thyroid gland in inbred strain A. From long-continued transplantations of tumors, we concluded that various mammalian tissues have the potentiality to immortal life, although the organism of which they form a part is mortal. It is not possible to repeat this condition with normal tissues through serial transplantations in the same way as with tumors, because in the case of normal tissues the differences between the individuality differentials of donor and host will, in all probability, be so great that the toxicity of the bodyfluids of the host and the aggressive action of its lymphocytes and connective tissue will injure and destroy the transplants within a relatively short time. On a former occasion we attempted to overcome this difficulty by using for serial transplantations in the rat a very resistant tissue, namely cartilage, and in this way it could be shown that parts of the cartilage, originally taken from an old rat, after transplantation into younger hosts may remain alive for so long a time that the total age of the transplant exceeds that usually reached by rats.

Subsequently it occurred to us that this problem might perhaps be attacked successfully in still another manner, namely, by serial transplantation in closely inbred strains or families, in which the individuality differentials in all the animals belonging to such a strain or famly had become identical or almost identical. Here, the reactions against the transplants on the part of the hosts should be lacking, or so slight that no serious damage would be inflicted. We have already reported on short serial transplantations of this kind in guinea pigs, but more favorable for this purpose seemed to be the inbred strain A mice, because under natural conditions the life of this mouse is shorter than that of a rat or guinea pig and because strain A was the one in which severe reactions against transplants from donors belonging to strain A would be least likely to occur. We selected for transplantation, the thyroid gland, with or without the parathyroid. In this case, not only isolated tissues, such as fibroblasts or epidermal cells spreading out diffusely, but a complete organ would be kept alive.

Ten experiments were carried out, in which one or two thyroid glands were transplanted from a strain A mouse through a number of generations of A mice, the donors in different experiments varying in age between 5½ months and 14 months. Three of the donors had reached the age of 14 months, one the

between the reactions against the different tissues transplanted from one single donor into the same host. It was also of interest that in cases in which an infection had taken place in a transplant between near relatives within an inbred strain, this infection and the reaction against the transplant usually remained localized and did not interfere with a good preservation of the tissue at some distance from the place of infection.

In *strain D*, a number of transplantations were carried out in which tissues were transplanted from parents to children, or from children to parents. In these experiments, also, autogenous or almost autogenous results were obtained, but the number of syngenesio-reactions, with marked lymphocytic infiltration, was distinctly greater than when tissues were exchanged between brothers and sisters. In some instances, even homoiogenous reactions were noted. On the whole, the reactions in transplantations from children to parents were somewhat less favorable than the reciprocal ones. Also, *in strain C57*, transplantations from parents to children and from children to parents gave, in the majority of cases, autogenous reactions, but there were several marked syngenesio-reactions. In seven transplantations from C57 children to C57 parents the average grade was 2.59. In strain A, autogenous reactions were obtained in transplantations from parents to children. In a general way, it seems that in these transplantations between mice in which the individuality differentials were very similar but not yet identical, lymphocytic reactions were more frequent than in transplantations between more distantly related mice. This corresponds to the fact that a lymphocytic reaction is especially prone to develop when the thyroid transplants are well developed, while in a stunted thyroid, such as we find especially after transplantations between more distantly related mice, the lymphocytic reaction is either lacking entirely or at least it is weaker.

Exchange of tissues between hybrids composed of two different inbred strains and between hybrids and parents as a test for their individuality differentials. In the beginning of this chapter experiments have already been reported in which we transplanted tissue of hybrids (C57xD)F_1 to mice belonging to different litters as well as to the same litter of the same kind of hybrid, and other experiments in which we exchanged tissues between hybrids and parents, and vice versa. A few experiments were also considered in which we transplanted tissues from hybrids, not to their direct parents but to other members of their parent strains.

In earlier investigations we had carried out, on a somewhat larger scale, similar transplantations in which we used hybrid strains (C57xA)F_1, (tan C57xA)F_1 and F_2, and (C57xC)F_1, as well as the reciprocal hybrids. It will not be necessary to describe these experiments in detail, because they gave essentially the same results as the transplantations already discussed. We shall, therefore, merely state the main results obtained.

In the following table, the results of transplantations discussed in the beginning of this chapter (Group I) and of this group (Group II) are shown. There may be added to the data contained in this table the fact that in control experiments, in which transplantations of tissues (thyroid, cartilage and fat

of repeated retransplantations be diminished; this may perhaps lead to a diminution of the injury inflicted on the grafted tissue.

It is very probable that a sclerosed thyroid does not produce thyroxin and is therefore functionally inactive, and it may be also that such a thyroid produces a smaller amount of the individuality differential substance; this condition would tend to diminish the invasion of the transplant by lymphocytes. Accordingly, we found the lymphocytic infiltration in these serial transplantations of thyroid gland either lacking or slight; but, the fact that the individuality differentials in strain A approach, although they do not quite attain, an autogenous state would in itself be sufficient to account for a lack of lymphocytes. However, in the preceding experiments we noticed that after a single transplantation of the thyroid gland, a sclerosed organ could be infiltrated quite markedly with these cells. There is reason for assuming, then, that in the case of the thyroid gland a deficiency in the amount of hormone produced by the host thyroid is not required for a successful transplantation of this organ, and, correspondingly, it is doubtful whether the diminution in function of the sclerosed thyroid renders its transplantation easier.

Transplantation between nearly related individuals in inbred strains of mice. If the inbreeding in mice had led to a completely autogenous condition of the individuality differentials among all the members of a closely inbred strain, there should be no difference between the results of transplantations between nearly related mice—namely, those which belong to the same litter and their own parents—and between other mice which belong to different litters. They all should show autogenous reactions. We carried out, accordingly, a considerable number of experiments in which we studied transplantations of various tissues, but especially of thyroid, cartilage and fat tissue, between near relatives, and those between brothers and sisters.

From these experiments it may be concluded that in brother-to-brother transplantations, autogenous reactions predominated in all strains, and to the highest degree in strains A, C3H and CBA; but syngenesio-reactions did occur, even in strain A, although they were more frequent in strains D and C57, and in the latter strain even one homoiogenous reaction was noted, with some increase in fibrous tissue. Variations in the age of the hosts within the range of 2 and 6 months did not seem noticeably to affect these results. The syngenesiotransplants were characterized especially by an increase in lymphocytes, which could destroy even otherwise autogenous tissue; but there could be associated with this reaction a slight increase of fibrous tissue. It was observed also that transplantations from one donor to two hosts, or from two donors to one host, might elicit somewhat different reactions. We note, then, that even in brother-to-brother transplantations a complete identity of the individuality differentials in these inbred strains has not yet been attained, and that again differences exist in this respect between different strains. But there are indications that in transplantations between litter mates the average of the reactions approaches somewhat more completely the autogenous type than in transplantations between non-litter mates; this seems to be the case, in particular, in strains D and C57. In all these transplantations there was, as a rule, a correspondence

enter, are in accordance with expectations, considering the fact that, in contrast to the homogeneous genetic constitution in the F_1 generation, in the F_2 hybrids, different individuals may differ in their genetic constitution.

Transplantation of ovaries in inbred mice and interaction between endocrine factors and individuality differentials. We have carried out a larger series of transplantations of the ovary in inbred mice for two reasons: 1) the ovary in the mouse offers certain advantages over other organs for the study of the relations between transplantation-reactions and the character of the individuality differentials, since this organ contains a variety of structures which differ greatly in sensitiveness and thus in their ability to survive; it presents a gradation in the degree of reactions between the individuality differentials of host and donor, without regard to the cellular response of the host against the transplant. The corpora lutea and large follicles are the most sensitive structures; they are followed in order of decreasing sensitivity, or of increasing resistance, by the medium, small and primordial follicles, by germinal epithelium and ducts derived from it, by medullary ducts, by cortical spindle cell connective tissue, and by interstitial gland. The Fallopian tubes, situated near the ovaries, are also rather resistant structures. By noting the survival or lack of survival of these different constituents of the ovary, we can grade the degree of similarity between the individuality differentials of host and donor; (2) the ovary, also on account of its structure, is a good test organ for the evaluation of the importance of endocrine factors, of age of donor and host, as well as of the sex of the host in the results of transplantation. We are especially concerned with the question as to how far the endocrine influences, which originate in the host ovaries, may affect the fate of the transplants. The associated structures of the ovary, such as germinal epithelial cysts and ducts, medullary ducts and interstitial gland, together with the Fallopian tubes, are comparable in their power of resistance to thyroid gland, striated muscle tissue and some other organs, since they are at least partially preserved under conditions in which the various types of follicles are in a graded manner destroyed. Because the transplantation of the ovary of the mouse has thus many advantages over that of many other organs in the analysis of the individuality differentials, we have carried out a large number of ovarian transplantations in various inbred strains of mice, but this account will be limited to a brief statement of some of the principal conclusions at which we have arrived.

In grafting the ovaries in strains AKA, Old Buffalo, and to some extent also in strain New Buffalo, the follicles are much more injured than are the associated structures of the ovary and the constituents of other organs usually used in our transplantations, indicating a difference in the constitution of the individuality differentials in the animals composing each of these strains. In the other strains, A, C3H, CBA, C57 and D, the disharmony between the individuality differentials is not so great that it affects the state of the follicles very considerably, although it may affect especially the formation of large-sized follicles and corpora lutea under conditions in which the preservation of small-sized follicles is not yet interfered with. In these latter strains, in

tissue) to strange strains (AKA, Old Buffalo and C) were made, as usual severe homoiogenous reactions approaching grade 1 were obtained.

The data in table I show a very good correspondence in group I and group II, and their significance is thereby increased. In addition to the conclusions already discussed, it may furthermore be stated that transplantations from parents to hybrids do not correspond to autogenous but to syngenesiotransplantations, and that transplantations from hybrids to parents seem to give somewhat higher grades than the average homoiotransplantations in not inbred strains, and inter-strain transplantations in inbred strains. These facts, together with the better results obtained in transplantations between hybrid F_1 brothers than in those between parents and hybrid F_1, suggest the presence of a considerable number of genetic factors as determiners of the individuality differentials, and they also confirm the conclusion that the strains which were used for hybridization had not yet attained a completely homozygous condition.

TABLE I

	Group I	Group II
Inter-strain transplantations	1.29	
Intra-strain transplantations	2-2.26-2.81	
From hybrid F_1 to parent strains	1.50	1.44
From hybrids F_1 to actual parents	1.42	1.48
From parents to hybrids F_1 (children)	2.60	2.54
From parents to hybrids F_1 (not actual children)	2.35	
From hybrids F_1 to brothers	3.04	3 or 2.75
From hybrids F_1 to other hybrids F_1 (not brothers)	1.91	

Besides these transplantations between parents and hybrids F_1 into which two inbred strains had entered, we carried out some experiments in which transplantations were made from the second generation of hybrids (black C57xA)F_2 and (tan C57xA)F_2 to their parents, which were hybrids F_1, and to their grandparents, C57 and A, and also the reciprocal transplantations; in addition we grafted tissues from some F_2 hybrids to their brothers. The results obtained may be summarized as follows:

(1) From (black or tan C57xA)F_2 to parent hybrids F_1: mostly syngenesio-reactions.

(2) From (black or tan C57xA)F_2 to grandparents (C57 or A): mostly homoio-reactions.

(3) From (black or tan C57xA)F_1 to hybrid F_2 children: mostly syngenesio-reactions (somewhat less favorable than those in 1). The reactions in (1) and (3) are intermediate between those from parents to F_1 hybrids and from F_1 hybrids to parents.

(4) From (tan C57xA)F_2 to brothers: results intermediate between syngenesio- and homoiogenous reactions (average grade 2.05). These are less favorable than those obtained in transplantations from hybrids F_1 to brothers. In general, the results in these various transplantations in which hybrids F_2

a 15 months old host, 2½ months after transplantation, good, large follicles and many well preserved corpora lutea were found and there was only a slight lymphocytic infiltration; likewise in a 19 months old host, large follicles were noted, but here there was too some lymphocytic infiltration around the ovary. We have found indications also that a lymphocytic infiltration may set in late, and further, that a number of successive transplantations may lead to injury of the transplant.

Such serial transplantations were carried out in strain A. The transplanted ovaries remained in each host for 4 to 6 months, after which time they were re-transplanted into another host. Altogether, the ovaries were thus kept in successive hosts for periods ranging between 14 and 24 months and the age of these ovarian grafts varied between 24 and 36 months. Only in four of eleven of these serial transplants was living transplanted tissue found at the end of the experiment; and only associated structures were recovered, such as germinal epithelium lining a cyst, ducts consisting of germinal epithelium, and interstitial gland tissue; in one instance, also a Fallopian tube with preserved epithelium, connective tissue and muscle tissue was found. Lymphocytic infiltrations were observed in some cases around or near surrounding parts. These experiments prove that only the most resistant tissues were able to survive, and they also indicate that the ovary is less suitable for such serial transplantations than the thyroid gland.

Transplantation of anterior pituitary glands as indicators of individuality differentials in inbred strains of mice. We have shown previously that anterior pituitary glands may be transplanted successfully, and that such transplants may exert effects on the ovaries, which, under certain conditions, increase the incidence of mammary gland carcinoma in the inbred strain A. Additional experiments have now been made, in which we compared the reactions against these transplants in various inbred strains differing in regard to the homozygous state which they had attained. In most cases, between two and six pituitary glands from sisters and brothers were transplanted subcutaneously.

In this series of transplantations, the transplants of anterior pituitary glands survived readily for long periods of time in most of the inbred strains, especially if the glands were taken from brothers and sisters. However, in the Old Buffalo strain, the transplants had apparently been destroyed at the time of examination: whether this was an accidental occurrence or is an indication of a more destructive action of the host against the transplant, perhaps caused by a greater dissimilarity of the individuality differentials, an effect similar to that seen after transplantation of the ovaries in this strain, needs further investigation. The most interesting observation from our point of view is the fact that under the conditions of these experiments, lymphocytic infiltration around or in the transplant was, on the whole, rare, and if it occurred at all it usually remained slight. In this respect the results differ from those obtained after long-term transplantation of thyroid gland, ovaries and adrenal gland, where as a rule the lymphocytic infiltration was more marked. On the basis of these experiments we may also conclude that the transplantation of anterior pituitary glands is less suited for the analysis of individuality differentials than that

which the individuality differentials have become more similar although not yet completely identical, accumulations of lymphocytes may still allow a grading of the different degrees of homozygosity which have been attained. The lymphocytes invade first the tissues adjoining the ovaries, and only later the ovary proper. Within the ovary the lymphocytes invade the granulosa of preserved follicles last; they seem to be especially attracted by the yellow vacuolated interstitial gland tissue, which corresponds closely, in some respects, to certain cell complexes found in the cortex of the adrenal gland in mice. In both of these organs vacuolated cells may act as phagocytes, neighboring cells may coalesce, or the nuclei may form central rosettes. By the combined use of the lymphocytic reaction and of the survival of the various types of follicles as tests for the individuality differentials, the strains can be arranged in the following order of decreasing homozygosity: (1) strain A; (2) strains C3H and CBA; (3) strains C57 and D; (4) strain New Buffalo; (5) strain Old Buffalo, and (6) strain AKA. In strain Old Buffalo, the best results were obtained if the ovaries were transplanted into ovariectomized sisters, but neither ovariectomy nor the close relationship between donor and host alone was sufficient to insure a good preservation of the follicles. However, in general there are conditions in which, with the aid of a hormone constellation that is very favorable for the survival or growth of the transplanted ovaries, the latter may be preserved and the follicles may grow even in transplants from different litters. This was the case after transplantations, for long periods of time, into castrated males: such mice offer, perhaps, the most favorable hormone-constellation, which may overcome the damage caused by a certain degree of incompatibility between the individuality differentials of host and transplant. Likewise in strain A, in which there is also a difference in the constitution of the individuality differentials between mice belonging to the same and to different litters, the removal of the ovaries somewhat improved the results.

While in strains New Buffalo and AKA transplantations into sisters seemed to have an advantage over transplantations into different litters, ovariectomy did not appear to be of much significance, the improvement, at best, being only slight. Transplantation into males gave at least as good results as transplantation into female mice, and after transplantation into older mice there was in quite a number of cases a survival of the ovarian structures. Furthermore, given favorable relations between the individuality differentials of host and donor, ovarian transplants may remain alive in the host for a length of time which is so great that the age of the ovary, or of some of its surviving structures, may exceed the average age of the individuals belonging to a certain strain. Thus, in a nonovariectomized mouse, young corpora lutea were found in a transplanted ovary which had remained in the host for about 18 months; here, lymphocytic infiltration had occurred. Also in strain A it was not difficult to recover well preserved ovaries which had been transplanted for half a year or longer. Moreover, in strains A and C57, ovaries were successfully transplanted into 12 to 20 months old female mice. In strain C57, donor and host belonged to different litters and the hosts had not been ovariectomized. In

here at very late periods. This fact again seems to confirm the conclusion that the individuality differentials in these inbred strains have not yet reached an autogenous condition.

Transplantation of the thyroid and parathyroid glands for longer periods of time in various strains of mice and the analysis of the individuality differentials. These experiments were made in addition to earlier transplantations of the thyroid gland, which we have reported in the preceding pages, and in the large majority of which the examination took place at earlier periods, usually between 12 and 30 days following transplantation.

In *strain A*, seven transplantations of thyroid and parathyroid, either alone or in combination with other organs, were made; examination took place between 9 and 15 months, mostly between 10 and 11 months, following transplantation. The thyroid and parathyroid glands were well preserved, although there was in some instances a small amount of fibrous tissue around some acini. In or around all transplants, except one, there was definite lymphocytic infiltration, which was moderate in some cases and marked in others. This also occurred in a case in which donor and host belonged to the same litter. We may then conclude that an autogenous relationship between the individuality differentials has not yet been reached and that the absence of lymphocytic infiltration at a given time, indicating apparent compatibility between the individuality differentials of host and transplant, does not actually prove such a harmonious condition; it merely indicates a lack of incompatibility great enough to cause a lymphocytic reaction at a particular time, but does not exclude the possibility that if the transplants had remained in the host for longer periods, such a reaction would have occurred.

In *strain D*, thirteen transplantations of thyroid and parathyroid were made; in all but four cases the organs from brothers and sisters were used. In two of the animals from different litters no transplants were found 9 months after transplantation. In the two remaining animals from different litters, 8 months and 20 days, and 4 months after transplantation respectively, the structure of the transplants was not like that of autogenous grafts and here was much lymphocytic infiltration. In the nine cases in which donors and hosts belonged to the same litter, the examination took place in most instances about 9 and 11 months after transplantation; in one, the time of examination was about 4 months, and in another it was 1 month and 3 weeks. In only two of these transplants, namely in those examined after 9 months, was the lymphocytic infiltration lacking; in seven grafts it was moderate or marked, but in every case quite definite. These experiments confirm then again the conclusion, that a homozygous condition did not exist in strain D. We may add that this is due not merely to an early branching-off of sublines from the main line, because late reactions occurred also between brothers and sisters, therefore between members of this strain, which have been propagated continuously and directly by brother and sister matings. In both strain A and strain D, antagonistic reactions of the hosts may thus develop against tissues which had been transplanted a considerable number of months previously.

In *strain C57* the grafts remained, in eight cases, from 2 to 8½ months in

of some other organs, because they elicit so weak a reaction on the part of the host and do not, therefore, make possible finer gradations of the individuality differentials in the various strains.

Transplantation of the adrenal gland and the analysis of the individuality differentials. While some of the principles concerning the nature of the individuality differentials have been established through the experiments on transplantation, which we have already discussed, still, it may be expected that an extension of the type of organs and tissues subjected to transplantation may give additional data. Such further information was obtained by means of transplantation of the adrenal gland.

As far as the analysis of the individuality differentials is concerned, the most important observation in these experiments concerns the lymphocytic infiltration, which was found to occur with increasing intensity around degenerating cortical tissue as late as eight or ten months after transplantation, while, on the contrary, well preserved areas of cortical tissue were not invaded by lymphocytes. This condition corresponds to the action of the lymphocytes of the host towards ovarian transplants from donors whose individuality differentials differed from those of the host. Here, also, the lymphocytes invaded mainly degenerating interstitial gland tissue and not at all, or only very rarely, the preserved granulosa of well developed follicles. Therefore, certain structures within several types of transplanted organs behaved not unlike autogenous transplants, while other structures, especially those undergoing a certain kind of degenerative change, behaved like homoiotransplanted tissue. We have already discussed the possible causes for these peculiar responses of different structures; the possibility exists that certain tissue differential substances may combine with strange individuality differentials to form substances whch attract the lymphocytes; or some types of growing or well preserved tissues may give off substances which protect them against an invasion by lymphocytes, which might otherwise occur if disharmonious individuality differentials interact; or lastly, it is conceivable that individuality differentials are produced or set free in larger quantity in certain stages of regression in various tissues. However, such a behavior of the lymphocytes is not usual. Thus, we have found very strong indications that well preserved thyroid tissue can be invaded and destroyed by masses of lymphocytes, if there is a slight divergence between the individuality differentials of host and transplant. In autotransplanted organs we have not observed, thus far, a marked invasion by lymphocytes of the degenerating cells, but this point is being investigated still further at the present time in the case of adrenal glands. In non-transplanted, autogenous adrenals frequently degenerative changes occur, similar to those which attract the lymphocytes in transplants, but they do not lead to intensive accumulation of these cells. The time at which accumulations of lymphocytes occur seems to vary in different inbred strains; it apparently takes place earlier in strains in which the differences between the individuality differentials of the various members of the inbred strains are as yet considerable. It is also of interest that pronounced infiltration with lymphocytes may be seen in transplants from sisters or brothers in closely inbred strains, but they occurred

preserved granulosa of follicles is not readily invaded by lymphocytes. An even more striking example of such differences is to be noted in the activity of different structures within the adrenal gland. Here, the degenerating yellow cortical tissue attracts lymphocytes in large masses, although this reaction in its full strength may occur only very late after transplantation; on the other hand, preserved strands of glomerulosa or fasciculata cells are not invaded by lymphocytes. Such peculiar differences in the attraction which various tissues exert on lymphocytes and which must be connected with peculiarities in the metabolism of these various tissues, may prevent a complete correspondence between reactions against different kinds of tissues which have been transplanted at the same time, from the same donor into the same host.

the hosts. The outcome of these experiments also indicates that there was a lack of a completely homozygous condition.

In *strain New Buffalo*, only those grafts in which donors and hosts were litter mates survived for greater lengths of time; lymphocytic infiltration occurred in these transplants

In *strain Old Buffalo* the transplantations carried out were fewer in number than in the other strains. As far as we can judge from these experiments, the individuality differentials in this strain are farther removed from the autogenous state than those in strains A, C3H, D, C57, and CBA. This conclusion would be in harmony with our findings in the related New Buffalo strain, although in the latter the reactions against transplants from other individuals, belonging to the same strain, was less severe than in the Old Buffalo strain.

On the whole our previous findings concerning differences or similarities between the individuality differentials in the various inbred strains of mice are therefore confirmed in these experiments.

In general, we may conclude from all our experiments with closely inbred strains of mice that, as with inbred rats and even with inbred guinea pigs, an autogenous state of the individuality differentials has not yet been reached, and that the degree to which this state has been approached differs in different strains. However, it is probable that the differences in the severity of reactions against transplants from other members of the same inbred strains are not entirely due to the different degree to which the genetic constitutions have become similar in the various individuals belonging to a strain; it may be due also to the differing intensity of the reactions of different hosts against a similar degree of disharmony between the individuality differentials in a given strain. Yet, after all, it is principally the differences in the genetic constitution between the various members of a strain and therefore also the differences in the individuality differentials of these animals, which determine the character of the reactions against transplanted tissues and organs. All these experiments add then new evidence for the conclusion that multiple factors determine the nature of the individuality differentials and that the time of the appearance of a reaction may be an indicator of the degree of disharmony between the individuality differentials of different hosts and donors; if the disharmony is relatively slight, the reaction may appear at a late date following transplantation.

A complication may be caused by the fact that various organs and tissues may differ very much in the intensity of the cellular reactions which they induce in the host. We observed formerly that cartilage is relatively inert and we attributed this characteristic to the relatively inactive metabolism in this tissue. We have now found that also anterior hypophysis is a tissue that remains relatively well preserved after transplantation and that calls forth no lymphocytic reaction, or only a slight one. This organ is therefore not well suited for the analysis of fine differences in the individuality differentials. Furthermore, we see that different structures in the ovary induce the lymphocytic reaction to a very different degree; the yellow interstitial gland tissue and also the corpus luteum tissue are quite active in this respect, while the

The connective-tissue reaction around the heterotransplants is in general very strong; there is a tendency for the connective tissue soon to become transformed into fibrous-hyaline tissue, which latter encapsulates the transplant and may surround some of its constituent parts and injure it through the exertion of mechanical pressure; but in certain heterotransplants, such as those of thyroid or kidney, where the tissues early become entirely necrotic, the ingrowth of the connective tissue cells was at first less marked than it is in those homoiotransplants where the reactions are severe; it seems that a heterogenous tissue, which is either completely necrotic or is near death, and in which the metabolism is therefore very weak or wholly suspended, may attract fibroblasts less actively than a more energetically metabolizing tissue which is giving off homoiotoxins.

In syngenesiotransplants and in some homoiotransplants large and dense masses of lymphocytes may invade and destroy the grafted tissue independently of a preceding activity of the connective tissue. Such a condition we do not find in heterotransplants. Here, lymphocytes may invade the graft usually only in association with the connective tissue of the host. This invasion of lymphocytes may, in the course of time, be quite marked; it may, however, remain slight or be lacking altogether if the heterotransplant becomes entirely necrotic at an early date, as often occurs when thyroid or kidney is transplanted; but even in these cases a considerable lymphocytic infiltration may later take place in the fibrous capsule surrounding the graft, or in the fibrous tissue adjoining it, or sometimes also in the fibrous-hyaline tissue that has replaced the graft, where it may exceed, in density, the infiltration found in the majority of homoiotransplants.

As stated, the appearance of larger numbers of polymorphonuclear leucocytes distinguishes heterogenous transplants from homoio- and syngenesiotransplants; these cells accumulate in and around the capsule, they may pentrate into the transplant and be found around or in the necrotic tissue; they may either be scattered or may form small accumulations, or even dense masses, in certain areas. Necrotic material seems to be their chief point of attraction. In and around homoiotransplants, on the other hand, leucocytes as a rule are noted only in the first three days following the operation when necrosis and changes in the circulation and in the permeability of vessels may be responsible for their appearance; they occur in these, and even in syngenesiotransplants of the mouse, more frequently in places where much fibrous tissue has been produced, and, above all, in fibrous tissue that has invaded and replaced fat tissue. The possibility exists that in the mouse we may have to deal with bacterial infection in those homoiotransplants in which leucocytes appear in larger numbers, and this raises the question as to whether also in heterotransplants the accumulation of polymorphonuclear leucocytes may not at least in part be due to contamination with bacteria. The presence of bacteria and their responsibility for the accumulation of polymorphonuclear leucocytes is suggested particularly also by the development, in some instances, of localized, abscess-like masses of these cells in or around the heterotransplants. Furthermore, the fact that if a piece of mammalian tissue is trans-

Chapter 10

Heterogenous Transplantation of Normal Tissues and of Blood Clots

WE SHALL NOW discuss the characteristic features of heterotransplantation. The marked toxicity of the bodyfluids, causes early injury and necrosis of the transplants, without the co-operation necessarily of cellular elements of the host. This necrosis affects different organs and different tissues with unequal rapidity in accordance with the degree of resistance of these structures, and even within the same organ or tissue there may be differences in the rapidity of necrosis, inasmuch as those parts which are in general more resistant in their constitution, or which, owing to their situation, are more protected against various kinds of injurious factors, succumb less quickly to the action of the heterotoxins. Organs or tissues which have a low degree of resistance, such as bone marrow, or a medium degree of resistance, such as thyroid, kidney, fat tissue, striated muscle tissue or epidermis, are destroyed by the heterotoxins within one or two weeks. In the skin, the hair follicles are more resistant than the epidermis proper, a fact which agrees with the observation that under certain conditions, for instance, after painting the skin with the carcinogenic hydrocarbon methylcholanthrene, the epithelium of the hair follicles shows a higher degree of resistance than other parts of the epidermis. Cartilage may survive for four weeks or somewhat longer, although in some instances it may undergo necrosis sooner. Thus, in transplantation from rat to guinea pig, necrosis of cartilage and perichondrium may be found after 20 days or even as early as after 12 days. Likewise, in the exchange of tissues between rat and mouse the greater part of the cartilage in one case was preserved as late as 25 days after transplantation, while in some other animals cartilage and perichondrium soon became entirely necrotic. Fat tissue as a rule was found necrotic very early, as for instance, after 6 days, and it was usually invaded by connective tissue cells and by small vacuolated cells, the latter evidently representing phagocytes, which took up fat in the form of small droplets. Some lymphocytes were observed admixed to the connective tissue and polymorphonuclear leucocytes were found frequently, sometimes in large quantities, sometimes only as scattered cells. There were certain heterotransplants in which no leucocytes were to be seen at the time of examination. After homoiogenous transplantation the necrosis of the fat tissue is, as a rule, less extensive than after heterotransplantation, the necrotic tissue is less actively invaded by connective tissue, and furthermore, under sterile conditions of operation the polymorphonuclear leucocytes are usually entirely lacking, except in the first few days after operation.

homoiotransplanted tissues, the latter, when killed by heating, no longer elicited the typical homoio-reaction. There was a marked lessening of the lymphocytic reaction normally called forth by homoiotransplanted thyroid or cartilage together with the adjoining fat tissue, but again the connective tissue reaction was not markedly diminished in such a thyroid transplant; it could even be slightly increased. The reaction of the connective tissue is partly directed against necrotic tissue; it is, therefore, not seriously affected by the heating. But, the much more specific lymphocytic reaction in case of homoiotransplantation depends upon the presence of actively metabolizing tissue, because homoiotoxins are produced and given off mainly by functioning homoiogenous tissue. This interpretation is supported also by other observations and experiments, to which we shall refer later. Of interest is also the finding of Siebert, that heating homoiogenous cartilage and perichondrium at 47° for 30 minutes seemed to increase the regeneration of cartilage by the perichondrium around necrotic injured cartilage. On the other hand, it might be argued that the heterotoxic action remains almost as strong after heating as it was in the case of unheated tissues, because the exposure to moderate heat did not seriously injure the contaminating bacteria; but against this interpretation may be cited experiments in which the heterogenous tissues were exposed to the temperature of boiling water. In these experiments, to which we have already referred, the thyroid and cartilage, with adjoining fat tissue, of rats, were boiled for 5 minutes in normal NaCl solution and then transplanted to guinea pigs. In the examination, which took place after 12 and 20 days, the reactions were found to be essentially the same as after heterotransplantation of the unboiled tissues, except that in the boiled thyroid the colloid of the acini remained preserved in the grafts, while, as was to be expected, the acinus tissue, as well as cartilage and fat tissue, was necrotic. The essential point in such experiments is that the boiled heterogenous tissues still attracted the polymorphonuclear leucocytes in large numbers, and that the infiltration with the latter was almost as strong as in the transplants of non-boiled rat tissues in the guinea pig; lymphocytes were in evidence in or around these tissues after 12 days, but they were no longer found after 20 days, although they were seen in the unboiled grafts at this time. It is very difficult to believe that under the conditions of these experiments bacterial infection was the cause of the accumulation of polymorphonuclear leucocytes in or around the transplants.

2. In further support of these conclusions, there may be cited experiments carried out by Blumenthal, to which we have already referred. When he transplanted small pieces of autogenous, homoiogenous or heterogenous tissues under the skin in various species, changes in the absolute number and distribution of lymphocytes and polymorphonuclear leucocytes took place in the circulating blood, which in principle corresponded to those occurring locally around such transplants. In the case of homoiotransplantation there was an increase in lymphocytes, which began in the first few days following transplantation and reached a maximum between about the third and tenth days. The exact time of the maximum varied with different tissues, according

planted into a frog for only a few hours and then re-transplanted into a mammalian host, large collections of polymorphonuclear leucocytes are attracted by it and then destroy the transplant, likewise suggests this interpretation. Similar is the result if pigeon skin is transplanted for various periods into the frog and then re-transplanted into the guinea pig. That necrosis as such, even necrosis of fat tissue, cannot be responsible for the accumulation of such cells in or around the transplant is shown by the observation that in rat and guinea pig, as well as in pigeon and chicken, necrotic areas do not noticeably attract polymorphonuclear leucocytes if the necrosis develops in homoiotransplanted tissues. And even in the mouse there are many homoiotransplants entirely free from leucocytic infiltration.

Various considerations, however, make it seem more likely that these cell accumulations are due to heterotoxins, which are given off by the graft and which diffuse into the surrounding tissue, especially after the graft has become necrotic. We must then assume that there are chemical differences between the necrotic areas in homoiogenous and in heterogenous tissues, which are responsible for the different modes of reaction of the polymorphonuclear leucocytes, and that the latter are attracted by either necrotic or living tissue, in contrast to homoiogenous tissues, which do not attract them, although a few isolated leucocytes may be found here also in the first few days following transplantation; however, the possibility cannot as yet be entirely excluded that the growth of microorganisms is promoted by conditions present in the heterotransplants, as compared to those in auto- and homoiotransplants, or that both these factors—microorganisms and heterotoxins—may be active. That a greater strangeness of the individuality differentials of host and graft may favor the accumulation of bacteria is shown especially in mice; when transplants come from nearly related donors, the collections of polymorphonuclear leucocytes usually remain localized at one spot, while similar collections in transplants from further distant donors often affect the transplanted piece as a whole, or at least over wider areas. As we have seen in the preceding chapter, together with the leucocytes, also connective tissue, lymphocytes, and, in the case of the fat tissue, small-vacuolated phagocytic cells, invade the homoiotransplanted mouse tissue. In our laboratory several experiments have been made for the purpose of deciding between the various possibilities regarding the appearance of polymorphonuclear leucocytes and some associated conditions.

1. Siebert exposed, in the water bath, the thyroid and cartilage, with adjoining fat tissue, of rats to temperatures ranging from 43° to 51°, for from 15 to 45 minutes, and then transplanted these pieces into guinea pigs; examination took place after 20 days. The activity of lymphocytes and polymorphonuclear leucocytes was only slightly decreased and the connective tissue reaction was even somewhat increased as compared with that observed in tissues not previously heated. We interpret these results as indicating that heterogenous tissues, even if they are killed through heating previous to transplantation, still possess and give off their specific heterotoxins to almost the same extent as the unheated tissues. When the same procedure was used with

count of bacteria living on the normal skin seemed to be higher during the hot summer weather than during other seasons. There was also some indication that the destruction of the bacteria proceeded more actively after heterotransplantation than after autotransplantation of the skin.

The results in all these experiments make it probable that the polymorphonuclear leucocytes were attracted to heterotransplanted tissue not mainly by bacteria attached to these tissues, but by the action of heterotoxins. While it seems that this is, in general, the correct interpretation, still, under certain conditions, and especially in transplantations carried out in the mouse, it may well be that slight infections with microorganisms play a certain role; but it is probable that even if microorganisms should be involved, they act in conjunction with toxins derived from the tissues and that they exert a greater effect when the individuality differentials of host and transplant differ greatly from each other than when they are closely related.

We may further conclude that while the typical reaction against syngenesio- and homoiotransplants occurs only if these tissues are alive and presumably actively metabolizing, the heterotoxins are present and active also in dead tissues. Additional evidence in favor of this conclusion is furnished by the results of experiments in which the reactions of a host against autogenous, homoiogenous and heterogenous blood clots were compared. If autogenous and homoiogenous blood clots are transplanted into the guinea pig, the characteristic differences that are found between the reactions of the host against autotransplants and homoiotransplants of living tissues, such as thyroid, kidney, epidermis, or cartilage and fat tissue, are almost or entirely lacking. This seems to be due to the fact that the cellular elements enmeshed in the net of fibrin soon die and no longer give off the autogenous and homoiogenous substances which bear the individuality differential. These clots are merely organized by the connective tissue and the blood vessels of the host like inert foreign bodies, no noticeable amount of homoiotoxins being given off after transplantation into another individual of the same species. As a result of the invasion by the fibroblasts of the autogenous or homoiogenous host, first a provisional organization takes place, representing a mixture of blood coagulum and of the cytoplasmic substances of the fibroblasts; subsequently, a definite organization is effected by continued ingrowth of fibroblasts. A few lymphocytes may be admixed to the capillaries and fibroblasts, which move into the clot, but they are not frequent. As stated, there are no definite differences between the autogenous and homoiogenous blood clots under these conditions. While phagocytes may, to a limited extent, be seen in homoiogenous blood clots, they are not a prominent feature in the process of organization. The phagocytic cells distintegrate into granula, which later help to form a hyaline material. Polymorphonuclear leucocytes are, on the whole, not conspicuous in these transplanted blood clots. It seems, then, that the nonnucleated erythrocytes included in the network of fibrin do not give off homoiotoxins to any noticeable degree. Much more pronounced was the reaction against heterogenous blood clots, such as that observed if clots were exchanged between rat, guinea pig and rabbit. In these cases, accompanying the

to the consistency of the pieces, which evidently determined the readiness with which the individuality differential substances were extracted from the tissues. After syngenesiotransplantation, the reactions appeared somewhat later, in accordance with the diminished toxicity of the substances given off by transplants of this type. The reactions to heterogenous grafts consisted in a primary increase in polymorphonuclear leucocytes in the circulating blood, which tended to appear a few days earlier than the reactions to homoiogenous transplants. After heterotransplantation of thyroid tissue, the maximum in the count of polymorphonuclear leucocytes was reached on about the fifth day, approximately two days earlier than the maximum observed after homoiotransplantation; however, the reaction sets in as early as on the 2nd or 3rd day after the operation, but then a regression in the number of leucocytes begins and on about the 10th day a normal count has again been reached. This is followed by a secondary reaction consisting in a relative and absolute increase in lymphocytes, which usually reaches a maximum between the 14th and 16th days and likewise is followed by a regression to normal. These reactions occurred in the guinea pig, rat and mouse with equal regularity. That they were caused by contamination with bacteria can be excluded, since, as a rule, no infection was found, and even where after homoiotransplantation a slight infection with bacteria had taken place, this did not call forth a noticeable increase in the number of polymorphonuclear leucocytes in the blood; such an increase was observed only in cases in which pus from an abscess, that had formed in and around the graft, had ruptured and escaped into the surrounding tissue. A small number of microorganisms did not therefore cause alterations in the number and distribution of blood cells, such as is seen after transplantation of heterogenous tissues. In these experiments as well as in those in which the local reactions around transplants were studied the results were, on the whole, constant and this fact again could not very well be reconciled with the assumption that bacterial contamination and subsequent growth of the bacteria—occurrences which are of an accidental character and therefore necessarily variable—were responsible for these changes.

3. A direct attempt was made to follow the fate of bacteria normally adhering to pieces of skin after its transplantation, in order to determine their possible role in the attraction of leucocytes. In these experiments, Ermatinger, Queen and Parker transplanted autogenous as well as heterogenous earskin pieces into the subcutaneous tissues; after 1, 2 or 3 days, they were removed for bacterial examination. Autotransplants of skin in guinea pigs, rats and rabbits were studied, as well as heterotransplants from guinea pigs to rats and rabbits, and also the reciprocal transplants. A progressive decrease in the number of bacteria in the skin pieces was found on successive days. The large majority of the microorganisms were destroyed within the first 24 hours, while after 48 hours nearly 25 per cent of the plates were sterile; after 3 days, sterile plates were found in 62 per cent of the cases and in the rest of the pieces the number of colonies was very small. Staphylococci survived longest. However, during the very hot season a considerable increase in the number of bacteria was observed in the first three days in several instances and the

that the differences in the local reaction and the reaction in the circulating blood is due to a greater sensitiveness of the latter, which allows the recognition of homoiogenous differentials in material in which these differential substances are present in so small a quantity that they can not be discovered by the local reaction. But in this instance we have again to consider the possibility that less specific reactions against non-living protein material may participate in these general reactions and that this factor may introduce a complication which is absent in the local reaction.

There are some additional questions concerning heterogenous transplantations which are of more general interest and which we shall now consider: (1) Does a relationship exist between the time of survival and the growth processes in heterotransplants and the reactions of the host tissue against the latter, on the one hand, and the phylogenetic relationship between host and transplant on the other? (2) What are the relations between growth processes in heterotransplants and time of survival? To what extent do regenerative growth processes take place in heterotransplants? (3) What differences occur in heterotransplantation of different organs and tissues? (4) Do the results of reciprocal heterogenetic transplantations differ and what is the reason for this difference? In order to answer these questions we may discuss briefly the principal results obtained in some of our series of heterotransplantations, while we omit a description of others.

Heterotransplantation of guinea pig skin. In association with W. H. F. Addison, we observed that after transplantation of guinea pig skin into other species the epithelial cells grew less actively than after homoiotransplantation, but the growth continued for some time, as indicated by the presence of mitoses in the epidermal cells. Mitoses were found in the rabbit as late as 8 days, in the dog, 7 days, and in the pigeon 5 days following transplantation. However, the mitoses were less numerous than after homoiotransplantation and the difference between the activity in the homoio- and heterotransplanted tissue increased with increasing time after transplantation. In heterotransplants the mitotic activity usually ceased a few days before the tissue became entirely necrotic; but, it happened that a mitosis could be seen near the time of death. The hair follicles, which are burrowed deep in the tissue and are surrounded by a connective tissue capsule, thus being most effectively protected, remained alive longest and showed the greatest number of mitoses. Also, the cells of the surface epidermis lived for some time and continued to produce keratin; the connective tissue of the host surrounded the transplanted epidermis; yet the growth energy of the epithelium was too weak to cause a cystic distention of the transplant from pressure of the newly produced keratin, in contrast to the finding after homoiotransplantation, where the epidermis does, as a rule, form a cyst. However, even the homoiotransplanted tissue may lose this ability if its growth energy has been weakened, as for instance, by previous serial transplantation. Lymphocytes migrated into the heterotransplanted epidermis from the surrounding host tissue and they, together with the pressure exerted by the fibrous capsule, helped to destroy the epithelium which had already been injured by the action of the heterotoxins.

connective tissue cells surrounding and invading the clot, were many lymphocytes and the capsule of the heterogenous clot was thicker than that of the homoiogenous clot. However, the thickness of the connective tissue capsule and the number of lymphocytes varied in different instances and even in different places in the clot. Areas of partial solution also were visible and a larger number of phagocytic cells was present in such heterogenous clots. The latter cells, which may show a xanthoma-like tissue arrangement, are able to dissolve particles of fibrin as well as the erythrocytes. Furthermore, the hemolysin present in the serum of the host and active against the blood cells of strange species may help in the solution of parts of the coagulum. Also, polymorphonuclear leucocytes, which again are much more prominent in heterogenous than in homoiogenous transplants, invade the clot and may aid in its destruction. These differences in reaction against different types of clots are quite definite, although the same technique was used in these various transplantations. In principle, the reaction against all kinds of heterogenous clots is about the same, although some minor quantitative differences may exist in different species; thus, in the guinea pig the solution of the clot proceeded somewhat more actively than in the rat and likewise the number of polymorphonuclear leucocytes in and around the clots was somewhat greater than in the rat. These experiments indicate, then, a noticeable similarity between the behavior of heterogenous blood clots and heterogenous tissues. In both cases, lymphocytes as well as polymorphonuclear leucocytes participate in the reaction in addition to the connective tissue, and heterotoxins are given off by non-living material in both types of heterotransplants; on the other hand, homiotoxins, are given off only by living tissue transplants, but not to any marked degree by the necrotic homoiogenous blood clots.

By measuring the lymphocytic and leucocytic reaction in the circulating blood, Blumenthal discovered not only differences between the reactions against homoiogenous and heterogenous blood clots, but he found also differences between the reactions against autogenous and homoiogenous clots, corresponding to those found against the corresponding normal tissues, in particular, he noticed an early increase in lymphocytes after homoiogenous transplantation of blood coagula. By means of this method he could show, furthermore, that also homoiogenous and heterogenous plasma clots may elicit the typical blood cell reactions, although they appeared somewhat later than the reactions following transplantation of the whole blood clot. It appears, then, that the individuality differential substances are present also in fibrin, although they may perhaps not be of the same kind as those present in the cells. The reactions affecting the white cells in the circulating blood seem to indicate the presence of substances in the blood which are carriers of the individuality differentials, although they do not elicit the local homoiogenous reactions. These reactions, which are called forth by fibrin and which presumably are present also in fibrinogen, are not induced by injections of blood serum; the latter does not apparently possess these individuality differential substances. However, it is possible that the individuality differential substances in cells included in whole blood clots are the same as those present in the fibrin, but

but some capillaries were noted between some of the acini. Lymphocytes were seen only occasionally in these places, being found especially where fibroblasts had invaded the transplant or were active around it, as well as in the capsule of the graft surrounding blood vessels. On the whole, heterotransplanted thyroid as such did not attract lymphocytes to any marked extent; indeed, these cells and the connective tissue contributed only secondarily and to a minor degree to the destruction of the graft.

In general, the connective tissue of the heterotransplanted thyroid became fibrous during the second week. The number of lymphocytes in the transplant itself was small, but in the course of the second week a marked accumulation of lymphocytes could take place in the surrounding capsule and at some distance from the thyroid proper; lymphocytes collected also in the fibrous tissue resulting from the organization of the necrotic material.

Heterotransplantation of kidney tissue into various species, studied in association with M. H. Myers, on the whole gave results similar to those found after heterotransplantation of skin and thyroid, but the duration of mitotic activity and the survival seemed to be slightly longer in the case of thyroid and kidney than of skin. A comparison of the period of survival and of mitotic activity in these various series of experiments is shown in the following tables.

TABLE I

Transplantation of Skin	Latest Time at Which Mitoses Were Seen	Time of Survival of Transplanted Skin Tissue
Pigeon to chicken	7 days	10–11 days
Pigeon to guinea pig	0 days	10 days (little tissue surviving)
Pigeon to rabbit	5 days	5 days (one piece 10 days)
Pigeon to frog	0 days	5 hrs.
Guinea pig to rabbit	7–8 days	10 days
Guinea pig to dog	6–7 days	7 days
Guinea pig to pigeon	5 days	10 days
Guinea pig to frog	0 days	1 day

TABLE II

Transplantation of Thyroid	Latest Time at Which Mitoses Were Seen	Time of Survival of Transplanted Thyroid Tissue
Guinea pig to rat	9 days	9 days
Rabbit to rat	9 days	11 days (in 1 of 3 pieces)
Rabbit to guinea pig	6 days	8 days
Cat to rat	11 days (a few mitoses)	14 (18?) days

Heterotransplantation of cartilage. After heterotransplantation of cartilage together with the adjoining fat tissue, connective tissue of the host invaded and largely replaced the fat tissue, but it was also able to invade the cartilage,

After re-transplantation of the guinea pig epidermis from the foreign species back to the guinea pig, the epithelial cells grew only very weakly and, on the whole, the farther removed the first host species was from the guinea pig, the shorter was the interval after re-transplantation during which mitoses appeared. No growth took place in the guinea pig skin after transplantation into the frog, which is so unfavorable a soil that a piece remaining longer than 3½ hours in this distant species, did not grow after subsequent re-transplantation into the original donor. Guinea pig skin which had been kept in the rabbit for 2 days was able to grow after re-transplantation into its own species, but pieces that had been longer in the rabbit died after re-transplantation; in the pigeon, pieces that remained less than 5 days could be successfully re-transplanted into the guinea pig, but not if they were left in the former species for a longer time. If we consider merely the duration of mitotic activity and survival after a single heterotransplantation, the order of compatibility for guinea pig skin was approximately as follows: (1) rabbit, (2) dog, (3) pigeon, (4) frog. But in general the differences between these species in these respects were not great, with the exception perhaps of the frog, which had a very injurious effect on the transplanted guinea pig skin. However, this order does not obtain in regard to readiness of re-transplantation of this tissue, because a primary transplantation from guinea pig to pigeon was less injurious to the graft than a primary transplantation to rabbit.

Heterotransplantation of pigeon skin. In principle, the results were similar after homoio- and heterotransplantation of pigeon skin to those found in the case of guinea pig skin, but there were also some interesting differences. Even after homoiotransplantation of pigeon epidermis the epithelial proliferation was found to be very slight, although epidermis and connective tissue remained largely preserved. While the homoiotransplanted guinea pig skin formed a cyst because of its continued proliferative activity and keratin formation, and while, for the same reason, a necrotic area in the guinea pig skin was rapidly replaced by new tissue, the pigeon skin did not give rise to the formation of such a cyst and reparation of necrotic tissue did not take place on account of the lesser growth energy in the transplanted pigeon epidermis.

Heterotransplantation of thyroid gland. Cora Hesselberg and the writer studied transplantation of the thyroid gland in various species. *(a) Thyroid of guinea pig to rat:* The heterotransplanted thyroid succumbed readily to the action of heterotoxins, remaining preserved for a short time only under the best of conditions. The primary injury of the graft by the bodyfluids of the host was quite noticeable as early as 3 to 5 days after transplantation. The number of mitoses was much diminished, but they still could be seen as late as 9 days after operation; this was also the latest time at which living tissue could be found. The epithelium was best preserved in the neighborhood of growing fibroblastic tissue and, conversely, growing epithelium seemed to attract the fibroblasts. The latter penetrated also between acini and had a tendency to form dense fibrous tissue, which compressed the acini and contributed to their destruction. The vascularization of the graft was very poor,

tilage in cases of homoiogenous and even inter-racial transplantation, is lacking in this type of heterotransplantation. There was seen only one instance of heterotransplanted cartilage in which at an early period a slight attempt at regeneration was apparently observed; but in this instance the interpretation was not certain. These findings indicate that even in cases in which structural appearances indicate the survival of the cartilage and perichondrium, these tissues are functionally and metabolically no longer normal or comparable to the corresponding homoiotransplanted tissues. Similarly, the nuclear multiplication, which is a sign of an abortive regeneration in injured areas of homoiotransplanted striated muscle fibers, is lacking in heterotransplantations; here, again, there was one possible exception observed at an early period following exchange of tissues between rat and mouse, and in this instance, likewise, the interpretation was doubtful.

Of special interest in these experiments were some differences which we observed between reciprocal transplantations in mouse and rat. The number of polymorphonuclear leucocytes, as a rule, was greater in the rat to mouse transplants than in the mouse to rat transplants; this corresponds to the frequent appearance of leucocytes in the mouse also in many cases of homoiotransplantation, especially in the fat tissue. On the other hand, in the mouse to rat transplants the fibrous tissue formation was more advanced, which may perhaps be due to the greater reactivity of connective tissue cells in the rat than in the mouse, which we had noticed likewise in homoiotransplantations. Similarly, the lymphocytic reaction was stronger in the rat than in the mouse; again this corresponds to findings in homoiotransplants. In the circumference of these heterotransplants the lymphocytic infiltration was in some instances even more pronounced than around homoiotransplants, although the invasion of the heterotransplant itself by lymphocytes was usually less than that found in many homoio- and even in some syngenesiotransplants.

Heterotransplantations from Peramyscus maniculatus to mice of strain C57 and the reciprocal transplantations. Although the number of experiments we could carry out in this series was much more limited than in other series of heterotransplantations, nevertheless the results were concordant and we are therefore able to draw some additional conclusions regarding heterotransplantations. The difference in reactions against the grafts taking place in reciprocal transplantations was evident again in these experiments. The results are of special interest also on account of the somewhat diminished severity, in certain cases, of the heteroreactions. In transplantations from C57 to Peromyscus there was almost complete destruction of the transplanted thyroid, the cartilage with adjoining fat tissue, and the striated muscle; this was noted as early as 8 days and was observed thereafter up to 20 days following the operation. Parts of cartilage were occasionally found preserved, and after 12 days even a mitosis was seen in a peripheral cartilage cell. Lymphocytes as well as scattered polymorphonuclear leucocytes were noted frequently in these transplants, and sometimes there were, instead of scattered cells, collections or even masses of leucocytes. The fat tissue was largely or entirely replaced by fibrous tissue. The grades were 1 throughout this series.

especially the necrotic areas. Fibrous tissue formed in larger quantity around the heterotransplant of cartilage than around that of thyroid or of kidney, probably because cartilage was less rapidly destroyed and its effect on the host tissue extended therefore over a longer period of time; however, a similar reaction could take place also around heterotransplanted thyroid and kidney

TABLE III

Transplantation of Kidney	Latest Time at Which Mitoses Were Seen	Time of Survival of Transplanted Kidney Tissue
Mouse to rat	9 days	11 days
Rabbit to rat	11 days	11 days
Rabbit to guinea pig	5 days	6 days
Guinea pig to rabbit	12 days	20 days
Guinea pig to cat	7 days	12 days
Cat to guinea pig	0 days	0 days
Guinea pig to pigeon	6 days	10 days
Pigeon to guinea pig	0 days	3½ days
Pigeon to rat	0 days	0 days

in the course of the second week. In accordance with the large amount of fibrous tissue produced especially around heterotransplanted cartilage and fat tissue, large masses of lymphocytes accumulated in the surrounding connective tissue at some distance from the graft; a similar reaction could also occur around other kinds of grafts, but it was observed more rarely in such tissues as thyroid, skin and kidney, as a rule, probably because these were destroyed by the heterotoxins more rapidly than cartilage.

Exchange of tissues between rat and mouse. In addition to the transplantations mentioned above, we carried out also heterotransplantations of tissues from rat to mouse and from mouse to rat, on the assumption that between these relatively nearly related species the reactions against heterogenous grafts might be less severe. However, we found that the reactions did not differ in severity essentially from those obtained in transplantations between the other species which we had tested. Transplantations from rat to mouse and reciprocal transplantations caused much more severe injury than homoio- and even inter-racial transplantations. Not only was the damage greater if we considered the average results obtained in a number of individuals, but in each individual case it was very great. Moreover, the individual variations which we have found between different homoio- and inter-racial transplants were almost absent in this series and this lack of variation applied to heterotransplantations in other species as well. Only the time of survival of the cartilage heterotransplanted into mouse or rat showed more marked differences; in some cases it became necrotic at an early period following the transfer into the heterogenous host, while in other cases it could survive for almost four weeks, or even somewhat longer. But the degree of this variability was more apparent than real, inasmuch as even under the best conditions the new formation of cartilage by perichondrium, which is found around necrotic or damaged car-

mammalian or avian tissues into the frog was very injurious, but it is doubtful whether this result was entirely due to distance of phylogenetic relationship; it is possible that bacterial infection played a role in this instance.

Evidently the heterotoxic action in general is so strong that all heterotransplanted tissues are near the threshold of destruction and the factor of phylogenetic relationship becomes thus of minor importance; under these conditions, a little more or a little less intense heterotoxic action may be of less importance than some other factor of a secondary nature. Thus there is some indication that the guinea pig may represent a host more unfavorable to certain heterogenous tissues than the rat or rabbit.

(2) As to growth processes in the heterotransplants, these were very slight, as might be expected in view of the injurious action of the heterotoxins. The mitotic activity usually ceased from one to a few days before the complete necrosis of the transplant occurred. The continuous destruction of the heterotransplant is not therefore compensated by a marked new formation of tissue. Heterotoxin prevents the full recovery of the tissues after transplantation and causes their death after a relatively short time. Correspondingly, certain regenerative processes which are found quite normally in cases of homoio-, and even in inter-racial transplantations, are lacking after heterotransplantation; this includes, for instance, the new formation of cartilage from the perichondrium, as well as the multiplication of nuclei in striated muscle tissue. In a few exceptional cases, at early periods, there were possibly some indications of weak regenerative processes, but the interpretation in these instances was doubtful. Only in transplantation from Peromyscus to mice of strain C57, restricted regenerative growth was noted within the first two weeks after grafting; in one case, even a mitosis was seen in a young cartilage cell, but here, also, the growth soon ceased. It seems that for the same reasons, namely, the interference of active heterotoxins, it is difficult for the host capillaries to make connection with the capillaries in the transplanted tissue and to use these preformed channels for the establishment of blood circulation in the transplant, the grafted vessels presumably dying soon after transplantation; furthermore, this factor may be responsible for engorgement of the surrounding vessels and for hemorrhages into and around the transplant. (3) The difference in the fate of different heterogenous tissues used, such as thyroid, skin and kidney, was very slight; they all behaved in almost the same manner after heterotransplantation; only cartilage was definitely more resistant, as it was also in homoiotransplantation. It follows from these observations that the method of heterotransplantation is not suited for the determination of species differences; serological tests are preferable for this purpose. In this respect, heterotransplantation differs from homoio- and syngenesiotransplantation, in which latter, especially the cellular reactions as a rule are very fine indicators of the degree of relationship between individuality differentials of host and donor and in this respect are superior to serological tests. Transplantation as a method for the determination of individuality differentials may be compared to a delicate balance, able to distinguish between fractions of a milligram but ill-adapted to the

The reactions were less severe against the transplants from Peromyscus to strain C57. Here the grades were better, especially in early periods after transplantation. At 8 days, the grades were 2−; at 12 days, they varied between 2− and 1+; at 15 days, the grades were 1 and 1+, and at 20 days, they were 1 in three cases and 1+ in one case. At 8 and 12 days, some preservation of thyroid tissue and also of muscle tissue was found and in the latter there was some nuclear proliferation; also at 15 days a small part of the thyroid was preserved, and, in one case, even at 20 days. The fat tissue was replaced by fibrous tissue or invaded by small vacuolated tissue. At 15 and 20 days the muscle tissue was necrotic and more or less organized by connective tissue. At 8 and 12 days, no lymphocytes but scattered polymorphonuclear leucocytes were seen in certain instances. At 15 days, besides variable numbers of leucocytes in one transplant, also some lymphocytes were present, whereas at 20 days, only leucocytes, but no lymphocytes, were noted.

We find, then, a definitely less severe reaction in cases in which C57 mice were hosts and Peromyscus were donors, than in the reciprocal transplantations. Lymphocytic infiltration in general was more common in the former experiments than in the latter. These findings bring, therefore, additional confirmation of the conclusion, that in addition to the relations of the individuality differentials of host and transplant to each other, the strength and mode of the reaction of the host against the transplant is also a factor which has to be taken into account and which may influence the results obtained. The type of reaction which a certain species or strain shows is also, in all probability, due to the inherited genetic constitution. It is furthermore of great interest that in strain C57, the reaction against heterotransplants of Peromyscus may not be stronger than those seen in a type of homoiotransplantations in which the individuality differentials of host and donor are very dissimilar.

On the basis of these observations, and of others which we cannot describe in detail, we may answer the questions raised in the beginning of this discussion. (1) As to a possible corespondence between the severity of heterogenous reactions in different combinations of species and the phylogenetic relationship of these species, the data given in tables 1, 2 and 3 indicate a relatively great similarity in all these species as to time of survival and mitotic activity. Both periods were relatively short and, in general, there was no very definite correspondence between phylogenetic relationship of donors and hosts and the fate of the transplants. There was very little difference between the results of experiments in which tissues of rodents were transplanted to other rodents and in those experiments in which tissues were exchanged between rodents and cats; in some instances, transplantations in the latter gave even better results than in the former.

Even exchange of tissues between mammals and birds, which represent two different classes, could give results not unlike those observed in transplantations between species as near as rat and mouse. Only in certain cases was there an indication of a shorter time of survival and mitotic activity after transplantation into different classes. Thus, pigeon skin fared better when grafted into chicken than into a mammalian species. Transplantation of

Chapter II

Exchange of Tissues Between Different Varieties or Races (Subspecies)

THE REACTIONS against heterogenous transplants, on the whole, are sharply defined and distinct from those against homoiogenous transplants; a transition to the latter is seen, however, in the grafts from Peromyscus to mice of strain C57. If we pass from transplantations of tissues between different species to transplantations between different varieties, races or subspecies, the results are different; these correspond to severe homoiogenous reactions. Such experiments were carried out with Gray Norway rats and two mutant races derived from them, mutant albino and Curly Coat; these mutations were discovered and their bearers propagated by Helen Dean King, at the Wistar Institute in Philadelphia. Five series of transplantations were made, each one representing a different type. I. Autogenous transplantation of thyroid and cartilage and adjoining tissues in Gray Norway rats; II. Syngenesiotransplantation between brothers and sisters in Gray Norway and the two mutant races; III. Intrarace transplantations between not nearly related individuals in each one of these three races; IV. Interrace transplantations from one of these races to another; V. Exchange of tissues between these three races and tame albino and hooded rats. Examination took place at 9, 12, 16 and 20 days following transplantation; however, at 20 days the number of experiments available for grading was not as large as at 16 days.

In the grading of the reactions in these experiments, the second set of grades was used. In autogenous transplantations in the Gray Norway rat the grade was 6. The average grades in the other experiments, together with the range of variations, are shown in table 1. The highest and lowest grades in each case represent the separate grades for thyroid only, because these grades are sharper than those for cartilage transplants.

From these results we may draw the following conclusions: (1) After autogenous transplantation in the wild Gray Norway rat, an injurious reac-

TABLE I

	12 Days	Range of Variation	16 Days	Range of Variation	20 Days	Range of Variation
Series II	4.42	5.5–3.4	3.3	5.2–2.3	2.05	3.6–1
Series III	3.15	5.5–1	2.55	4.8–1	2 20	3.9–1
Series IV	2.60	4.3–1	2.15	3.5–1	1.80	3.8–1
Series V	2.30	4.2–1	2.	3–1	2.15	3–1

determination of differences in weight amounting to pounds; similarly, homoio- and syngenesiotransplantations distinguish between the finer degrees of relationship of individuality differentials, whereas, heterotransplantation is not quite adequate for the finer and more general distinction of species differentials.

(4) The data given also demonstrate that reciprocal transplantations, in which the role of donor and host are reversed, may differ as far as the fate of the transplant is concerned. We have referred already to the greater severity of reactions found in some instances in the guinea pig than in the rat, and we have also shown the difference in reactions in reciprocal rat-mouse and C57-Peromyscus transplantations. Similar differences in reciprocal transplantations may be found also in some instances in homoio- and in syngenesiotransplantations. It is the host which reacts against the graft, but the latter does not seriously affect the condition in the host; the ability to react strongly against a strange tissue differs in different species, strains, and probably even individuals.

There is one last conclusion which is of more general interest. In the case of syngenesio- and homoiotransplantations we find a relatively wide range of reactions in different individual experiments, in accordance with the great range of variations in the combinations between different individuality differentials of host and graft. In contrast to these, the range in the results of heterotransplantations is rather narrow; this is due, at least partly, to the great severity of the reactions in heterogenous transplantations, which approaches a threshold already in the relations between nearly related species, but it may also be due to the fact that the range of variation in the genetic constitution of the species differentials is much smaller than that of the homoiogenous differentials.

fest, the principal injurious effect being that exerted by the cells of the host. If the bodyfluid was injurious in a given host, it acted on the various organs and tissues from the same donor in a corresponding manner. These various tissues from the same donor must therefore all have possessed the same individuality differential; otherwise the bodyfluid from the same host could not have acted on all of them in this way. If we consider in a general way the cellular reactions of the host against the transplants and the structural changes in the latter, in these various series, we find that the degree of necrosis in the grafts and the degree of the substitution of the necrotic tissue, by fibrous tissue as well as the extent of the invasion of living tissue by fibrous tissue, became greater with increasing distance in relationship between host and donor. Likewise, the lymphocytic infiltration was the more marked the greater the distance in relationship between host and graft, provided the difference between the organismal differentials was not so great that it led to extensive necrosis of the graft and largely to the replacement of the necrotic issue by fibrous tissue. Such a graded replacement by connective tissue and also by lymphocytes could be followed especially clearly in the transplanted fat tissue; but on the whole, the lymphocytic infiltration was more marked around and in the thyroid than in the cartilage-fat tissue transplants, and as a rule there was a correspondence between the reactions against the thyroid and cartilage-fat tissue. In autogenous transplantation of the thyroid in Gray Norway rats the connective tissue tended to be loose, fibrillar-cellular rather than fibrous-hyaline, the blood and lymph vessels were prominent in the center of the transplant and marked lymphocytic infiltration was lacking.

While the reactions in transplantations between different races in rats varied in intensity in individual cases, they still fell within that part of the spectrum of reactions which characterizes homoiogenous relationship; but within the homoiogenous range of the spectrum they were situated at the end farthest removed from autogenous relationship. Notwithstanding the considerable degree of individual variations in the intensity of the reactions, the best grades attained in the interracial series did not equal the highest grades reached in typical homoiotransplantations; but there is no sharp break in these cases between the character of homoiotransplantations and of interracial transplantations, such as is found if we pass from homoiogenous or interracial transplantations to transplantations between nearly related mammalian species.

In a former smaller series VI in which thyroid, cartilage, fat tissue and bone were transplanted from white rats to cream or hooded rats and the examination took place after the grafts had been kept in the host for 20 and 21 days, the transplants of the thyroid gland were all destroyed or only a few acini were found, and these were compressed by connective tissue and in process of destruction by lymphocytes; likewise in the fibrous tissue as well, that had replaced the destroyed thyroid, there was still some lymphocytic infiltration. In the cartilage transplants the fat tissue was infiltrated or mostly replaced by fibrous tissue and there were variable amounts of lymphocytic infiltration. Perichondrial regeneration of cartilage around necrotic cartilage was

tion against the transplant was absent. (2) In series II-V with increasing length of time during which the grafts were kept in the hosts, the damage inflicted on the transplants became greater; this might be expected, provided a discrepancy in the individuality differentials existed between hosts and donors; it did not occur in autogenous transplantations where, on the contrary, signs of injury due to the operation became less and disappeared in the course of time. (3) If we consider the different series, we find that the intensity of the reactions on the whole increased with increasing distance of relationship between host and donor. This is most clearly indicated by comparing the grades at 12 and 16 days, and especially also by comparing the combined grades at 12 and 16 days. The latter were as follows: series II, 3.9; series III, 2.9; series IV, 2.4; series V, 2.2. At 20 days, the difference between these four series had almost disappeared, either because at that time the injury had become very marked in all of them, or because of the smaller number of experiments that were available for the determination of the average grade. The combined grades for thyroid at 20 and at 16 days also suggest an increasing deterioration in the preservation of the transplants with increasing distance of relationship. In these series, and especially in series III, IV, and V, the reactions were relatively severe and accordingly, grade I was frequent in individual transplantations. The reactions were not so strong in some instances in intrarace transplantations, although in other cases they also were severe. This is especially interesting in view of the fact that some of these strains had been inbred for a considerable number of generations, although not necessarily by brother-to-sister breeding; strain Gray Norway, for instance, having been inbred for 45 generations. Although the rats belonging to the same strain were closely related, still, even in series II and III the reactions were, on the whole, severe. (4) While the differences between the grades in the different series seem to be real, they were much less than one might have expected, considering the great differences in relationship between the rats in these different series. It is noteworthy that the reactions even between brothers and sisters were very marked and that they approached much more closely the reactions against distant donors than those against autogenous transplants. We must assume that the individuality differentials even in brothers and sisters showed quite definite differences and that these were great enough to cause injurious reactions against the grafts. At the same time, it appears probable that the sensitiveness of the host and his ability to react against even relatively slight differences in the constitution of the individuality differentials were great. (5) The transplantations within the mutant albino race showed less severe reactions than those within the other mutant race. It is possible that the mutant albino rats reacted less strongly than other mutant races against the individuality differentials of rats belonging to their own strain. (6) There were definite indications that also in rats the bodyfluids reacted on the transplants in accordance with the relationship between transplant and host, and that the reactions of the host cells were superimposed upon the effect of the bodyfluids. However, in many cases the damage done to the graft by the latter was very slight, or not mani-

plantation, grade 1 signifies the complete destruction of a tissue of medium sensitiveness, such as the thyroid gland, and the complete or at least very extensive substitution of fat tissue by fibrous tissue, the cartilage being preserved entirely or in part. But while in heterotransplantation the tissues are injured to such an extent that regenerative growth does not as a rule occur, this may take place in interracial transplantation. The reactions designated by grade 1 have, thus, a certain latitude, signifying both the less severe and the very severe injury inflicted, respectively, by interracial and by interspecies (hetero) transplantations.

only rarely seen. If we use the second type of grades, the average grades in these two last named series was 1.75 and 1.5, respectively. In ordinary transplantations among white rats, carried out at the same time, the average reaction was 2.8. In this series of interracial transplantations, also, there was a correspondence between the degree of genetic relationship between donor and host and the degree of the reactions against the transplants. Regeneration of cartilage by perichondrium was inhibited, but not entirely prevented. The bone marrow became necrotic and was replaced by fibrous tissue at an early date, while in homoio- and syngenesiotransplantations the marrow could remain preserved for a longer time; in agreement with the findings in other experiments, cartilage remained as a rule preserved, at least in part.

In series VII in the mouse, we carried out transplantations of thyroid, cartilage and fat tissue from wild gray mice to mice belonging to the inbred A and Old Buffalo strains. In general, the results corresponded to homoiogenous reactions, which were more severe in the series in which Old Buffalo mice were the hosts. In this series the thyroid transplant had been destroyed in animals examined later than 12 days. In the fat tisssue there was more and more ingrowth of connective tissue, as well as of vacuolated phagocytes, and after 16 and 25 days there was much infiltration with lymphocytes. In the mice from the A strain the thyroid was present up to 20 days, but it was stunted or incomplete and the organization of the necrotic center proceeded only slowly. Here, also, more and more connective tissue and vacuolated cells grew into the fat tissue, and, in some cases, the lymphocytic infiltration was quite marked. The transplanted bone marrow became replaced by connective tissue in all instances. However, in several animals some muscle fibers with nuclear chains were found in both strains of mice. There is, then, no sharp demarcation between these experiments and others in which homoiogenous tissues elicited severe reactions; the grades varied between 2— and 1, and the latter grade was obtained in the mice examined at the later dates. On the whole, there was a remarkable correspondence in the reactions against different tissues from the same donor in the same host. This applies to all of these transplantations and it comes out clearly, for instance, in series III, the intraracial transplantations. Polymorphonuclear leucocytes were not found, as a rule, in these experiments; if seen at all, they appeared especially in the fat tissue.

The main result which emerges from these investigations is, then, the demonstration that the reactions against tissues from different races or subspecies correspond to very severe homoiogenous reactions, and that they differ from heterogenous reactions in several respects. Furthermore, inasmuch as the race, Curly Coat, differs from Gray Norway in one single mutation, it may be concluded that such a mutation may have a definite effect on the individuality differential.

If we compare these transplantations between individuals belonging to different races with those in which donor and host belong to different species, the grades applied do not completely correspond to each other. This is particularly true of grade 1, the severest grade. In both of these types of trans-

transplantations in fowl. But in all these experiments, as well as in subsequent ones, the principal problem was the study of the conditions which permit successful transplantation and of those which prevent it. However, a second problem soon became prominent: transplantations of organs with internal secretion were used also in order to determine the effects of certain hormones on the growth and functions of various tissues and organs. As examples of transplantations of the latter kind, the experiments of Steinach on the feminization of male guinea pigs and rats, by implantation of ovaries into castrated males, and on the masculinization of female guinea pigs by the grafting of testes, and those of C. A. Pfeiffer on the effect of transplantation of testes on the endocrine function of the anterior pituitary gland may be mentioned. Both of these investigators carried out transplantations into litter mates and into very young animals. Steinach used castrated guinea pigs, and Pfeiffer, non-castrated rats, as hosts. In the latter experiments, the proportion of testicles in which the tubules survived was relatively great. In both series of investigations we have therefore to deal with syngenesio- rather than with homoiotransplantations. The analysis of individuality was not the principal objective in the large majority of these experiments. A further consideration of all the numerous experiments in transplantations which have been made during the last fifty or sixty years would therefore not contribute much to a fruitful analysis of individuality, and it is not needed because résumés of these investigations have already been given by various authors.

However, we shall here attempt, if possible, to find the main factors which caused the differences in the results in transplantations of tissues obtained by various investigators, and in particular the differences in their interpretation of these results. One of the principal differences concerns the question as to whether transplants may survive after homoiotransplantation, and whether they survive as well after homoiotransplantation as after autotransplantation. As already mentioned, especially in earlier investigations the view is frequently expressed that various homoiotransplanted tissues or organs survive as well as autotransplanted ones; but this view occurs also even in the more recent literature. This may be due (1) to the lack of differentiation between real homoiogenous and syngenesious transplantations, the latter succeeding better than the former; (2) to the disregard of the age of the transplants; it seems that organs from newborn donors can be more readily transplanted than organs from older donors, and likewise, that the reactions may be milder after transplantations into very young than into older hosts; (3) often to the lack of a complete microscopical examination of the transplants. The smaller transplants should be cut into serial sections and many sections should be available for study from the larger transplants. It is necessary that all important stages, from the beginning of transplantation until the reaction is definite, be examined in succession, and that the various modes of reactions on the part of the host—cellular as well as bodyfluid reactions—be considered and evaluated in as quantitative a manner as possible; and lastly, it is important, if there is a limit to the periods when examinations can be made, that such stages be selected as would permit the recognition of the presence of

Chapter 12

The Problems and the Criteria of Success or Failure in Transplantation of Tissues and Organs

AFTER HAVING stated the principal experimental data relating to the individuality and species differentials in higher organisms, obtained by means of transplantation of tissues, we shall now add some brief considerations concerning various problems which arose in the course of these investigations and the criteria of success or failure used in the evaluation of such experiments by various authors.

In the later period of the last, and at the beginning of this century, it was noted by some clinicians and pathologists that autotransplantations of certain organs may succeed better than transplantations into other animals belonging to the same species. Thus Knauer, Ribbert and others obtained more favorable results after autogenous than after homoiogenous transplantations of the ovaries, but Ribbert believed that in some instances also homoiotransplantations of organs may succeed. We found that tumors could be successfully autotransplanted in cases in which homoiotransplantations failed. We carried out successful autotransplantations of pigmented skin in guinea pigs into defects in white skin, but Carnot and Deflandres, who had obtained similar results, thought that homoiotransplantations succeeded equally well. However, Sale who compared the results of auto- and homoiotransplantations of pigmented skin in the guinea pig in our laboratory found that only autogenous transplants healed in permanently while homoiogenous grafts were as a rule cast off after some time and that during this preliminary period lymphocytes collected underneath the transplant. Christiani (1900-1905) believed that the thyroid gland in various species can be transplanted successfully into the same animal, as well as into other animals of the same species, and even into different races and varieties. Yet, the experimental immunological and serological studies, which began to develop actively during this period, had already exerted a certain influence on the interpretation of experiments in transplantation, and, accordingly, Christiani noted that transplantation into different families, orders and classes of animals did not succeed, with the exception of transplantations between guinea pig and rabbit, which were successful. But, somewhat later it was more generally recognized that homoiotransplants of organs did not, as a rule, survive. Halsted, for instance, obtained negative results with homoiotransplantation of parathyroid in dogs. In many cases at this time and also for some time afterwards, investigators did not definitely distinguish between homoio- and syngenesiotransplantation, although in other cases such a distinction was made, as for instance, by Goodale, who carried out ovarian

differential substances are given off into the circulation. Blood vessels respond to changes in the environment in certain respects similarly to the connective tissue. With the latter, they move into necrotic tissue or into foreign bodies soft enough to permit penetration by capillaries. But while the connective tissue is stimulated more by homoiogenous than by autogenous differentials, the blood capillaries are inhibited by the former and attracted more actively by the latter. On the other hand, contact with autogenous tissue tends to prevent the change of cellular connective tissue into fibrous tissue.

However, tissue reactions can occur also between adjoining tissues of autogenous constitution and these reactions may be altered if a homoiogenous, takes the place of the autogenous differential. Thus we have referred to the changes which are seen when pigmented skin is autotransplanted into defects in white skin in the guinea pig, or conversely, if white skin is transplanted into a defect in pigmented skin; the pigmented epidermis grows into the unpigmented epidermis and this process continues for a certain time until new boundaries are produced between these tissues. In the normal quiescent state of the tissues, each adjoins the other without reaction; but whenever a tissue disturbance takes place, such as is caused by the injury connected with transplantation, a struggle ensues between the two adjacent types of epithelium. The pigmented epidermis is the stronger one, but under normal conditions its superiority is merely potential; it becomes activated under certain conditions which disturb the tissue equilibrium, and then a reaction occurs against tissue elements possessing the same individuality differentials. If a syngenesio- or a homoiogenous differential takes the place of the autogenous differential, this reaction is suppressed The strange individuality differentials injure the tissue metabolism, as indicated by the loss of pigment, which may occur in homoiogenous or syngenesious pigmented epidermis. The degree of inferiority of the unpigmented epidermis and the mode of reaction of the tissues towards each other, if disturbances take place, may vary in different species, even in nearly related ones. In some instances, changes in the tissue equilibrium between pigmented and white skin in the guinea pig may apparently arise spontaneously in non-transplanted skin. Such an effect was observed by Saxton, Schmeckebier and Kelley, presumably under conditions in which a disturbance of the tissue equilibrium was due to some hidden metabolic change.

As stated, the reactions above described were found in the guinea pig. In the mouse, the transplanted pigmented skin does not extend into the adjacent tissue, probably because the chromatophores here are not epidermal. Also, in the tadpole conditions are, in certain respects, different. Thus Rand and Pierce noted that while white transplants of ventral tadpole skin to pigmented dorsal skin were invaded by the adjoining pigmented host epidermis, this could occur in autogenous as well as in homoiogenous transplants, yet, an individuality differential was also involved in this reaction, as is indicated by the fact that in many instances in autogenous grafts the desequilibration was not sufficient to cause an invasion. An injury by homoiotoxins may then have to be added, in order to overcome the inertia of the pigmented epidermis

Chapter 13

The Effects of Various Extraneous Factors on the Activity of the Organismal Differentials

THE REACTIONS of hosts against transplants possessing individuality differentials which show various and graded degrees of similarity or difference from those of the hosts, have been described; also the differences in the reactions noted in different kinds of tissues, these differences depending upon an interaction between tissue differentials and individuality differentials. Furthermore the differences in the action of hosts belonging to different species have also been analyzed and we have seen that such species differences may affect the reactions which take place in the host against strange individuality differentials. In the course of these discussions, various problems of wider biological significance have been introduced and these will now receive further consideration.

(I) *The interaction between tissues possessing different individuality differentials, and the interaction between tissues possessing the same individuality differentials but different tissue differentials.* We have seen that organisms react against strange individuality differentials by means of their bodyfluids as well as of certain cells and tissues, or the latter may be the predominant reacting agents. The reaction of the bodyfluids is the more specific one of these two types. The tissue reactions as such are not entirely specific, but they may become so if we take into account also the quantitative factors in their activity, in particular the intensity and time of their action, and also the interaction between different types of tissues and cells involved. The connective tissue in general reacts very readily against various kinds of changes in its environment. It reacts wherever cells and tissues in the neighboring area are injured or killed; also against dead foreign bodies and it is influenced in its behavior by variations in the activity of neighboring epithelial structures; but in addition, connective tissue reacts very finely to differences in the individuality differentials in the adjoining tissues, discerning here the slightest differences and responding in accordance with a definite time curve. With advancing age the connective tissue stroma undergoes changes similar to those induced by strange individuality differentials. The lymphocytes too react primarily in a non-specific manner against foreign bodies and against injured tissues, provided these changes do not exceed a certain intensity. It is the polymorphonuclear leucocytes which are activated whenever acute changes of a relatively great intensity occur; these cells become prominent as soon as the difference between organismal differentials has attained such a degree that the tissues are markedly injured, as, for instance, when tissues possessing different species differentials adjoin each other, or when species

Chapter 14

Hormones and Individuality Differentials

The organism is an approximately equilibrized mechanism in which the maintenance of the structural autonomy of the various parts and the integrity of the whole organism depend upon the inherited characteristics inherent in these parts, in particular upon the nature of the interacting tissues and their individuality differentials; furthermore upon their state of sensitization, and upon the degree of stimulation these structural units receive especially by hormones. Interaction of all these factors with one another takes place, including the interaction between individuality differentials and the hormones, and it is this last type of interaction which is of interest also in our analysis of the individuality differentials. In this interaction the endocrine function may be considered as the primary factor and we may inquire how this function would be affected by changes in the individuality differentials, or, on the other hand, the individuality differentials may be considered as the primary factor and we may inquire into the effect which changes in hormone action may have on the activity and efficiency of the individuality differentials, especially under conditions in which two different individuality differentials oppose each other.

Hormones as such, within a wide range of their action do not possess individuality differentials and are independent of the latter, but the organs in which they are produced and the tissues upon which they act carry these differentials, and the new formation of tissues which takes place as the result of the function of certain hormones, may be greatly influenced by the nature of the individuality differentials of these tissues and organs; these effects may be unfavorably affected by the presence of other than autogenous individuality differentials. Thus we have seen that the formation of placentomata, which is controlled by the interaction between the corpus luteum hormone and mechanical stimulation, requires the presence of autogenous individuality differentials if the maximum effects are to be achieved, whereas, the presence of a homoiogenous differential has an inhibiting action on such processes. Likewise, the grafts of endocrine glands, such as ovaries and thyroid, develop and function best in a perfect autogenous environment.

On the other hand, under some conditions the presence or absence of certain hormones may affect the function and growth of organs and tissues in an environment in which the individuality differentials are not entirely adequate; or the action of hormones, also, may be of significance even in the survival of autogenous transplants. However, in this respect different organs seem to differ as to the degree to which they are influenced by the activity of certain hormones. In the organs which we have studied, the ability of hormones to prevent altogether the injurious effects of not quite adequate individuality

and to make its potential superiority actual. In adult rana pipiens, after transplantation of autogenous white skin to a pigmented dorsal area, no change in the condition of the transplant takes place, but following homoio-transplantation, the pigmented epidermis invades the white epidermis after about two weeks (H. H. Vogel).

A related process may take place at the border between the squamous epithelium of the cervix and the cylindrical epithelium of the uterine horns. This is especially noticeable in the mouse under the influence of stimulation of the vagina-cervix-uterus by estrogen. Under these conditions the squamous epithelium dominates over the cylindrical epithelium and begins to invade and replace it; it may also push into uterine gland ducts and here undermine or exert pressure on the cylindrical epithelium. This invasion may extend to various distances. There are indications that such changes may take place to a slight extent even without the hormone stimulation, but it is the latter which greatly intensifies the potential superiority of the squamous epithelium of the cervix over the cylindrical epithelium of the uterus.

In certain respects, also, cancerous growth may be considered as a related phenomenon. In this condition one tissue, as a result of long-continued stimulation, gains the ascendancy over adjoining tissues of the same or of a different kind and then begins to invade them. But in cancer such a change is not temporary, as in the examples previously mentioned; it is a permanent change, leading ultimately to the destruction of the whole organism. The mode of stimulation of one tissue which brings about this result in cancer may be of various kinds, but this is the less important factor; it is the reaction of the stimulated tissue which is characteristic. Thus we may conceive of an organism as an equilibrized system, composed of many mosaic parts, which function in harmony with one another. Various kinds of changes may disturb their equilibrium and then a potential tissue superiority may become actual. Tissue differentials, without the co-operation of individuality differentials, may condition such disharmonious reactions, but antagonistic, disequilibrizing reactions may be induced also by individuality differentials, and in some cases they are brought about by an interaction between tissue and individuality differentials.

stimulation of the thyroid gland in the guinea pig; they should therefore promote the growth of thyroid transplants and help the latter to overcome the injurious effects which a disharmony between the individuality differentials of host and transplant exert on the survival and growth of the latter. However, Martin Silberberg, who carried out such experiments, noticed that the hypertrophy of the transplanted organ is less readily accomplished than that of the non-transplanted thyroid gland, a finding with which our own is in agreement and which can be understood if we consider the less favorable circulatory conditions in a transplanted organ and a certain inadequacy in the relations between stroma and transplanted parenchyma whenever a disharmony exists between the individuality differentials of host and transplant. Silberberg furthermore made the interesting observation that a thyroid gland, rendered hypertrophic previous to transplantation by injections of anterior pituitary extracts, can be less readily successfully transplanted than a non-hypertrophic gland. Apparently the state of hypertrophy corresponds to an increased differentiation of the tissue, which makes the organ less resistant to the injury inflicted during the process of grafting. But on the whole, this investigator found favorable effects of injections of anterior pituitary extract on the homoiogenous thyroid if the injections were begun after the thyroid had been transferred to the new host. However, the results of the experiments of Silberberg, as well as our own in similar experiments, showed a certain variability, and on the whole, in the large majority of transplantations the action of the thyroid-stimulating hormone was not able to overcome the unfavorable results of homoiogenous transplantation of the thyroid gland in the guinea pig. These results agree with those of Bayer and Wense, who showed that injections of pregnancy urine, containing prolan, did not exert a beneficial effect on intra-ocular, homoiogenous transplants of testicle in the rabbit.

There is another condition in which hormones might possibly affect the fate of the transplant, namely pregnancy. In pregnant guinea pigs the reactions against homoiogenous transplants of thyroid, cartilage and fat tissue were severe in the majority of cases, even during early pregnancy of the host, but in some animals the reaction was relatively mild. It was conceivable that pregnancy exerts its effects by causing undernourishment of the transplants. However, in control experiments, in which young guinea pigs with an initial weight of 195-225 grams were underfed for a period of 18 days, so that the end weight was between 160-185 grams, the grades of the homoiogenous thyroid transplants were similar to those in well-fed animals, or they were even somewhat better in the underfed guinea pigs.

As already mentioned on the basis of experiments with transplantations of the thyroid gland, Christiani (1900-1905) stated that an endocrine deficiency is needed for the successful transplantation of an endocrine organ. He attributed this favorable result of a diminution in the amount of the endocrine organ, in particular the thyroid present in the host to the improvement in the vascularization of the graft in animals deficient in the production of the thyroid hormone. However, he actually observed merely an increased size and

differentials was not very striking; if such power existed at all, it was usually slight and in other cases it was lacking. Thus we observed that if ovaries were transplanted in certain inbred strains of mice, the unfavorable effects of relatively slight deviations from the optimum conditions of the individuality differentials could to some extent be remedied by using ovariectomized or castrated hosts; but in other strains, these improvements, due to the removal of the hormones secreted by the sex glands of the host, were lacking; in no instance were the effects very striking. We saw, furthermore, that multiple transplantations of anterior hypophysis succeeded in mice in which the host's own hypophysis was present and functioning, and that these transplants could exert certain hormonal functions and remain alive for considerable periods of time. This result was obtained in cases in which the disharmony between the individuality differentials of host and donor was only very slight; but there were some indications that when the differences between individuality differentials were greater, the transplants did not long survive. Likewise in experiments with thyroid and parathyroid glands, it was possible to transplant these organs successfully into hosts whose individuality differentials differed only to a slight degree, without first removing the hosts' own thyroid and parathyroid glands, and even several glands transplanted simultaneously in such hosts could survive. In the case of the adrenal gland of the mouse, such transplants degenerated to a large extent; but in many instances some part of the cortex survived for a long time if there was no marked disharmony between the individuality-differentials of host and donor.

It is possible to test the interaction between endocrine effects and individuality differentials in still another way, namely, by experimental administration to the host of an excess of certain hormones, in order to determine *whether this counteracts or accentuates the effects of not quite adequate* individuality differentials. Christiani, it seems, was the first to express the opinion that the need of thyroid tissue on the part of the host organism determined the fate of the thyroid transplants. In thyroidectomized animals the grafts healed in better than in those possessing their own thyroids. On the other hand, administration of thyroid substance caused an atrophy of the thyroid transplants. We were unable to observe any marked effect of the oral administration of thyroid tablets on the fate of thyroid transplants; it certainly did not prevent their survival in transplantations which otherwise would have been successful. At most, this procedure may perhaps have reduced the functional and mitotic activity of the thyroid transplant, as well as that of the host's thyroid, without, however, interfering very noticeably with the action of the individuality differentials, which latter determined essentially the success or lack of success of these transplantations.

Likewise, Carroll Smith did not notice that administration of potassium iodide had any marked effect on the fate of autogenous or homoiogenous thyroid transplants in guinea pigs. Also, experiments in which injections of extracts of cattle anterior pituitary were made into guinea pigs carrying thyroid transplants should have some bearing on this problem. Such extracts contain the hormone which causes a very marked growth and functional

lopian tube in rabbits (Guerriero), and of vagina in guinea pigs (Reynaud), show that stimulating hormones influence here, also, the growth processes and size of the transplants, without being needed, however, for the survival of the latter. In regard to the transplantation of ovaries, according to Lipschutz the total number of preserved follicles in the transplants is greater in ovariectomized than in non-ovariectomized animals, an effect which was observed however only under certain conditions and not as a general rule in our experiments; moreover, the interaction between individuality differentials and hormones were not considered by this investigator. Numerous experiments have demonstrated the effects of the anterior hypophysis on the ovaries and testicles, demonstrating the significance of the hormones given off by this organ for the growth and for the functioning of various constituents of the sex glands, and these influences extend also to the transplanted sex glands (Engle, Moore and Price, Takewaki, Bayer and Wense, and others). If we consider the results of these investigations as well as of our own, on which we have already reported, and those of Pfeiffer, we may conclude that the following conditions are involved in the survival and growth of transplanted sex glands: (1) The relation between the individuality differentials of host and transplant (genetic factors); (2) The age of the transplant; the transplantation of the sex glands of newborn animals seems to succeed better than that of older animals, at least in the case of the testes; (3) The removal of the sex glands of the host; this favors, in the first place, the growth and function of the transplant, and it may, secondarily, affect also its survival; it seems to be a more important factor when the hosts are older animals; (4) Transplantation to the opposite sex; there are some indications that under certain circumstances this may be a more favorable procedure than transplantation to the sex of the donor. The order in which these factors are cited indicates probably also their relative importance, the first one being the most important.

The following are the principal conclusions concerning the relation of variations in hormone actions to the fate of transplants. The effects of administration of hormones to the host, or of a diminution in the amount of hormones in the host on the fate of transplanted organs varied in different organ transplants and perhaps also in analogous organ transplants in different species. They were moderate, slight, or perhaps entirely lacking in some experiments with thyroid, parathyroid and ovarian grafts, or they were quite pronounced, especially in the case of adrenal grafts. They were found in transplants of endocrine organs, as well as in transplants of other organs or tissues on which hormones may exert a certain influence. Such effects may be noticeable after autotransplantation as well as after homoiotransplantation. In the former, it may be assumed that continued hormone stimulation prevents the gradual atrophy which might ensue due to the combined effect of lack of function of the transplanted organ and of injurious conditions existing at the site of transplantation. Homoiotransplanted organs, as a result of the increase in growth momentum acquired with the aid of hormone action, are enabled, to some extent, to overcome the unfavorable conditions caused by disharmonious

hyperemia in transplants in completely thyroidectomized hosts, as compared to animals in which a part of their own thyroid glands had been left intact. Haberer and Salzer (1909), in rabbits, and more recently, Ingle and Cragy, in rats, likewise noted a better vascularization and better growth of the acini in completely thyroidectomized animals than in those in which a part of the thyroid had remained. Halsted (1909), in the case of the parathyroid gland, found in dogs a better growth of autogenous parathyroids in animals in which their own glands had been completely extirpated; he believed that an endocrine deficiency is necessary not only for the better development, but also for the survival of an autogenous graft. However, this opinion is based apparently on a relatively small number of cases, in which the autogenous parathyroid transplants were recovered in parathyroidectomized dogs, and this investigator is very cautious in stating his conclusion.

There are, however, experiments with transplantation of other endocrine organs, in which the effect of an endocrine deficiency on the survival of transplants of such organs is greater. Thus the experiments on the grafting of adrenal cortex by Wyman and Tum Suden, by Ingle, Nilson, Higgins and Kendall, as well as those of Lux, Higgins and Mann, showed that successful transplantation of the adrenal cortex depends, in the first place, upon the genetic relationship between donor and host, better results being obtained in inbred rats in litter mates than in less nearly related animals. Furthermore, transplants from newborn rats are more favorable than those from older donors, but in addition, a deficiency in adrenal hormone production in the host stimulates the growth of the transplanted gland very much. The influence of the hormone consists primarily in an enhancement of the growth processes in the transplant, but in addition there is strong evidence that this factor may favor also the survival of the graft; and again, as a result of the stimulation of the transplant by the specific hormone, the vascularization of the graft is improved.

We may add here some related observations concerning the influence of hormones on the transplantation of non-endocrine organs. In our earlier transplantations of the uterus in various stages of the sexual cycle in guinea pigs, we found a favorable effect of hormones given off by the host on the survival of the transplanted decidual cells. Jacobson, in autotransplanting the endometrium of rabbits into the pelvic cavity, observed that the different periods of the sexual cycle in the animal may affect favorably or unfavorably the fate of the transplant and that ovariectomy carried out at the time of the transplantation does not prevent the formation of endometrial cysts, but that it diminishes the size of the cysts and the thickness of their walls. Neumann noted that intra-peritoneal autotransplantation of endometrium succeeds if the animals possess their own ovaries, but that it is unsuccessful in previously ovariectomized rabbits, which indicates that the atrophic uterine mucosa cannot maintain itself in the host. Likewise, while transplantation of the uterine mucosa into sisters is successful, it does not succeed in brothers for the same reason. In these experiments we have to deal chiefly with autotransplantation.

Corresponding investigations concerning autotransplantation of the Fal-

hormone which is able to stimulate the transplant. These hormones act primarily on the parenchyma of the graft, stimulating its increased growth and function, and such increased activity of the glandular tissue may secondarily stimulate the activity of the stroma and especially also its vascularization, which then further aids the growth and power of survival of the graft.

It may therefore be stated that hormones may influence in various ways the effects of the individuality differentials on the fate of a transplant. By stimulating growth processes in the latter, hormones tend to neutralize the damaging action of strange differentials, and by depressing these growth processes they may intensify the effect of unfavorable differentials.

individuality differentials. But in all these experiments, the degree of strangeness of the individuality differential of the host was a factor in determining the effectiveness of the hormonal stimulation on the survival and growth of the transplant; if the disharmony between the individuality differentials of host and transplant was diminished, the results were better. The increase in growth momentum acts in these instances in principle in the same way as this factor acts in tumors, which thereby also are enabled, to a certain extent, to overcome the injurious action of non-adequate individuality differentials by an increased growth momentum. In some organ transplants, factors inherent in the transplanted cells seem to predominate over the endocrine factors, and such transplants are largely unaffected by an increase in hormone stimulation; or a minimal amount of such stimulation may be sufficient and its effectiveness cannot be increased by additional amounts of hormones. As stated already, in addition to these factors, the age of the host, and especially of the transplant may affect the results.

In earlier investigations concerning these problems emphasis was laid on the importance of the creation of a deficiency in the amount of the corresponding endocrine organ of the host, as far as the success of the fate of the graft was concerned. But in reality, there is hidden behind this diminution in the amount of the host endocrine organ an increase in the stimulation of the transplant by an increased function or an increased production of the effective hormone in the host. We may illustrate this interpretation by a reference to the conditions noted by us in the development of compensatory hypertrophy of the thyroid gland in the guinea pig. We found that this depends upon the balancing of two hormone effects. In the first place, the administration of thyroid hormone tends to diminish the growth and hormone production of the normal thyroid gland of the treated animal. The thyroid hormone given off by the intact thyroid gland, correspondingly tends to limit these activities. On the other hand, the thyroid-stimulating hormone of the anterior pituitary gland opposes this effect by stimulating the thyroid gland and by causing hypertrophy. The normal condition of the thyroid gland is the consequence of a certain equilibrium between these two opposing tendencies, and this equilibrium can be changed by suppressing or enhancing one of the two factors involved. By excising a considerable portion of the thyroid gland, the amount of thyroid hormone given off is diminished and, correspondingly, the stimulating activity of the anterior pituitary gains the upper hand; on the other hand, an increased activity of the thyroid causing a depression in the function of the anterior pituitary has the opposite effect. Somewhat similar considerations apply to the equilibrium in the thyroid gland, ovary, and perhaps also the islands of the pancreas and some other organs with internal secretions. It is not certain in these cases whether the intrinsic hormone, as for instance, the thyroid hormone, acts directly on the organ in which it originates or whether it acts on the controlling endocrine gland, the anterior pituitary. In the case of the ovary, estrogen exerts its effects evidently by way of the anterior pituitary. We may therefore conclude that the results of extirpation of an endocrine organ depend on the consequent surplus function of another

tissues or organs in the same organism have in common a chemical characteristic, which differs from those present in every other organism. These differences are genetic in origin and are therefore proportional to the genetic relationship between different individuals, but they are not identical with the genes. Such individuality differentials must be distinguished from the tissue differentials, which are the same in the corresponding tissues in different individuals, but which differ in different tissues of the same individual. Also, the red blood corpuscles possess these individuality differentials. At first, by means of the study of the fate of transplanted blood clots, and especially of the cellular reactions of the host against them, merely the presence of heterogenous, but not of homoiogenous, individuality differentials could be definitely established, but subsequently, by the use of the white blood cell reaction, Blumenthal could demonstrate also the presence of homoiogenous differentials in blood clots, and correspondingly, in the erythrocytes included in these clots, or at least reactions were found similar to those elicited by homoiogenous differentials present in the various tissues.

Following the experiments of Ehrlich and Morgenroth, who succeeded in producing hemolysins for homoiogenous erythrocytes in goats, Todd extended these investigations and obtained similar hemolysins for homoiogenous red corpuscles in cattle and sheep. In 1911, he found differences between red blood corpuscles of individuals in certain species by using the differential absorption method. These antigenic differences between the erythrocytes of individual animals could be readily demonstrated, except in cases where there was a close relationship between two individuals. In 1930 and 1931, this investigator prepared polyvalent homoio-(iso) agglutinating immune sera against fowl erythrocytes, and again, by using the differential absorption method, he found that the red blood cells of each individual examined differed from those of every other individual. Also, members of the same family differed from one another in the agglutinogens of their blood corpuscles, the degree of difference varying very much in individual cases. The investigations of Todd on antigens present in the erythrocytes of cattle were recently confirmed and extended by Ferguson, Stormont and Irwin, who, by repeated injections of one individual with the erythrocytes of another individual of this species, prepared homoiogenous hemolytic sera, or by injecting a rabbit with such erythrocytes, they prepared heterogenous hemolytic sera; they furthermore analyzed the antigens present in the erythrocytes in an individual animal by means of these two kinds of sera. These tests were refined by absorption of the antibodies from the immune sera by the erythrocytes of individual animals. Thus, these investigators found thirty different antigens giving origin to immune hemolysins. Each individual differed from all other individuals tested in the combination of the antigens present in its red corpuscles. There were indications that other antigens might be added to this number. There were moreover, strong indications that each separate antigenic substance was determined by a single dominant gene and that correspondingly many multiple genes determined the set of antigens in a certain individual. It is very likely that by using for immunization other species in addition to the

Chapter 15

Individuality Differentials and Blood Groups

TOWARDS THE end of the last century and in the beginning of this, serological methods had been established which made possible the distinction of species and wider groups of organisms. It was natural that the question should have been raised as to whether it might not be possible to distinguish races and even individuals belonging to the same species by these means. Thus Bruck believed that by the use of the complement fixation method it was possible to distinguish between different human races. Landsteiner, in order to find individual differences, studied the interaction of blood serum and erythrocytes in man and thus discovered the existence of four primary blood groups, which are based on the possession or lack of possession of the agglutinogens A and B in the red blood corpuscles and of specific agglutinins for the four types of erythrocytes. Subsequently it was found that also certain animal species possess similar blood groups and that there may even be an identity of some of these antigenic factors in the erythrocytes of different species, as for instance, of man and certain apes. A comparison of the distribution of these blood groups in different human races showed that the proportions of the four blood groups differed in different races, but that the blood groups which did occur were always the same. It was furthermore established that the interaction between the agglutinins in the blood plasma or serum and the blood-group factors is responsible for thrombi which form in the blood if blood transfusions are made in case the donor and recipient belonged to different blood groups. There was a definite analogy between the blood, with its cells and the complex protein-containing medium surrounding these cells, and a tissue in which the cells were separated by intercellular substances.

Previous to the serological investigations which led to these discoveries, surgeons had noticed a difference in the results of tissue grafting, in particular of skin grafting, if the latter was made into the person from whom the skin flap was taken, or into other individuals, and this observation suggested the presence of chemical differences in the constitution of these tissues in different individuals. Similar differences were found in experimental transplantations in animals and also, as we observed, in the transplantations of tumors; we interpreted these differences as being due to the specific biochemical relationships between the bodyfluids and the tissues in donors and hosts. In continuation of this work, begun in 1909, finer methods were developed for the investigation of the relationship between such tissues. These depended largely upon the study of the cellular interactions between tissues of the host and of transplants. On the basis of these investigations, gradually the theory of the individuality differentials developed, according to which all

slight differences may lead to weak and often long delayed reactions. Especially significant in this connection are transplantations within closely inbred strains; here the great difficulty in making the genetic constitution, which determines the nature of the individuality differentials, identical, is clearly demonstrated; it is also shown that the reactions against the latter are parallel to the differences in the genetic constitutions of host and donor and that these differences show all kinds of gradations. Taking all of these facts together, the only conclusion possible seems to be that many genes take part in the determination of the nature of the individuality differentials.

It seems therefore most probable that a further anlysis of the antigens present in erythrocytes will show that they are either identical with or are a part of the factors which call forth the individuality differential reactions against all kinds of strange tissues. The peculiar character of the erythrocytes makes it possible to obtain for the analysis of the constitution of these cellular elements, special hemolytic and agglutination reactions, which cannot be applied generally in the study of the reactions against strange individuality differentials; but even in the case of the erythrocytes, as a rule it is not possible to demonstrate the existence in the serum of the host of preformed hemolysins or agglutinins for the red blood cells of the donor. Other characteristics which we have discussed repeatedly make it possible to distinguish the individuality differentials of cells and tissues in general, and there is, therefore, no reason why the individuality differentials, which were established by entirely different methods and which are common to all tissues, should be subordinated to the factors which determine the agglutination and hemolysis of erythrocytes, which latter represent very specialized modes of reaction between particular kinds of cells and particular constituents of the blood serum.

These conclusions as to the relations between the antigenic constitution of the erythrocytes and the individuality differential do not necessarily apply if instead of the numerous antigens of the erythrocytes, we consider merely the four primary blood groups. By means of these it is possible to distinguish between certain individuals, and it is the identity or lack of identity of the blood groups to which two individuals belong that determines the compatibility of their blood in transfusions; as stated the blood shows some analogies to tissues; therefore the compatibility of the blood might be taken as an indication of the compatibility of the tissues comprising an individual. In pursuing this trend of thought, several investigators went still further and considered the blood group characters of an individual as the most significant features of his constitution.

The experimental data on which the evaluation of the correctness of this interpretation has been based were obtained in a comparative study of the results of skin grafting, in cases in which donor and host of the graft belonged to the same or to different blood groups. The number of skin graftings in man, in which the blood group relations between donors and hosts have been considered, is great, but the conclusions arrived at by various investigators differ very much. There are those who believe that the success of homologenous skin transplantations is determined by the blood group relations be-

rabbit, and by testing erythrocytes of various cattle with the normal sera of individuals from different species, a considerable number of additional antigens may be found in cattle erythrocytes. There is therefore no basis for the suggestion made by these authors that each one of the thirty genes, corresponding to the thirty antigenic substances found so far, might be located in one of the thirty chromosome pairs which the cells of cattle are supposed to possess. Inasmuch as these antigenic (individuality differential) substances are genetically determined, it is to be assumed that, considering the relatively large number of these substances, on the average there should be a greater similarity between the sets present in parents and offspring than between the sets of less closely related individuals. Experiments were in agreement with this postulate. And there is thus, in this respect, a correspondence between the effects of these multiple factors present in erythrocytes and the individuality differentials of tissues in general.

In the meantime, Landsteiner and others, by studying further the agglutination reactions in blood of man and of other species, had added several additional factors (P.M.N., Rh., A_1 and A_2) to the original A and B which were found in erythrocytes; accordingly, the number of factors which have to be considered in selecting compatible donors and receivers in transfusions of blood has also increased, and it seems very probable that this number will be still further increased in the future. As to the different agglutinogens which are present in and distinguish the red corpuscles of fowl, Kozelka, who also extended the work of Todd, believes it possible that their number is small, notwithstanding the infinite number of individuals which all differ in the character of their erythrocytes. This opinion is based on the fact to which Landsteiner and Wiener have repeatedly drawn attention, namely, that there are a great many possible combinations of relatively few factors. On the other hand, it must also be remembered that the greater the number is of different rabbit immune sera and of natural sera from different species which are used for testing the agglutination and hemolysis of the corpuscles of individual fowl or mammals, the greater will become the number of factors which distinguish the erythrocytes of individual organisms. While it is true that theoretically a limited number of factors would suffice to account for a large number of differences between different individuals of a certain species, this does not necessarily prove that the number is actually very small.

As far as the individuality differentials are concerned, there are strong indications that the number of distinguishing factors is very great. We have referred already to some experimental data which strongly support this conclusion, such as the many fine gradations found in the strength of the reactions against syngenesious and homoiogenous tissues, in accordance with the relationship of donor and host, and the fact that no autogenous reaction is found against homoiogenous or syngenesious tissues except when we weaken artificially the ability of the host to attack the strange transplant, but that autogenous reactions occur only if the host and transplant possess the same genetic constitution. Furthermore, marked differences between the individuality differentials of host and graft lead to rapid and strong reactions, and

to the action of tissue coagulins extracted from the macerated kidney tissue. The further possibility has to be considered that in this case toxic substances which were peculiar to the duck kidney or also to other duck tissue were extracted from the implanted material; such toxins would in certain respects be comparable to special poisons which are present in some amphibian tissues. But substances of this kind are distinct from the species differentials. Considering all these data, there is no reason to attribute the effect observed by Sandstrom to the organismal differentials of the duck, but the substances responsible for it may represent a special kind of tissue differential.

Returning to the consideration of the blood groups, the conclusions stated above apply directly to the four primary blood groups; they probably apply also to the secondary factors (A and A_1, P.M.N., Rh) more recently found. All these factors determine the agglutination in vitro of blood corpuscles of one individual by the serum of another, as well as the results of transfusions of whole blood or of plasma. But as stated, it is very likely that additional factors will be discovered in the future also in human blood, and that the human erythrocytes contain individuality differential constituents. It is conceivable therefore that in some cases, in which transfusion of blood or of plasma has led to injurious reactions, strange individuality differentials may have been involved

It may then be concluded that the greater the number of factors which are found as determiners of the agglutination and hemolysis of the erythrocytes, the greater will be the probability that some, or even all, of these factors may also be constituents of the individuality differentials and thus may be the same as the factors which determine the interaction of tissues from different individuals. However, if we restrict ourselves to a consideration of the four original blood groups, which at first were the only ones known and analyzed from this point of view, these cannot, in all probability, be identified with the individuality differentials. This conclusion is in agreement with the experiments which we have already cited, as well as with some other data which may briefly be mentioned: (1) Brother and sisters may belong to different blood groups, whereas, many entirely unrelated individuals of the same species, and even members of different species, may belong to the same blood group. This identity of blood groups in members of different species does not improve the outcome of the corresponding heterotransplantations, which depends on the character of organismal differentials. The individuality differentials are graded according to the genetic similarity between the bearers of these individuality differentials. (2) There is much evidence that homoiogenous individuality differentials in no case have become, through inbreeding, absolutely identical with antogenous differentials. Even after many generations of brother-sister inbreeding there is still no complete identity of the individuality differentials in members of the closely inbred strains, and there are strong indications that reactions against slight disharmonies of grafts may appear in the hosts a long time after the transplantation has taken place. Furthermore, the strength of the response of the host against different organs and tissues differs and by selecting an active organ and the proper time for

tween donor and host; there are others, as, for instance, Lexer and Holman, who were unable to discover a relation between the compatibility of blood of donor and host and the result of grafting, and there are still others who do not hold that identity of the blood groups makes the homoiogenous transplantation of skin fully successful, but who still find indications that the sameness of the blood groups at least delays the destruction of the homoiogenous grafts.

In appraising the value to be attached to these divergent results, it seems that surgeons, with the largest experience in skin grafting, and among them those who have carried out these transplantations more recently, have obtained as a rule entirely negative results, while especially some earlier workers, with more limited experience, who did not follow the fate of the transplants over longer periods of time, believed that they had obtained confirmatory results; as to the latter, however, there always remains a doubt as to whether the definite healing-in of the transplants was actually seen, or whether the transplants were not gradually replaced by the adjoining skin of the host in cases in which there was compatibility between the blood groups of donor and host of the graft. More convincing then these earlier experiments are the experiments of transplantations in animals; especially the very careful investigations of Kozelka, in which skin of fowl was used for transplantation, are significant in this respect; no relation between the agglutinogens present in the erythrocytes of host and donor and the success of the transplantation was noted. Likewise in the work of Ingbrigtsen, who transplanted segments of arteries in cats, and that of Haddow, who transplanted sarcoma in fowl, the findings were independent of the agglutination reactions between the blood of donor and host. We believe, therefore, that the evidence available at present makes very improbable a direct relationship between the four primary blood groups and the individuality differentials of host and donor. Correspondingly, we must conclude that there is no definite correlation between the results of transfusion of blood and those of homoiogenous grafting of skin. There seems to be no more reason for assuming that the particular genes determining the four blood groups determine also the fate of homoiogenous transplants than for believing that the identity of heterophile antigens, among different classes of animals, makes heterotransplantation between these classes possible.

However, in recent experiments, Sandstrom made some observations of a different nature, which suggest to him a relation between organismal differentials and blood group antigens. Implantation of a piece of macerated metanephric tissue of the duck on the chorio-allantoic membrane of the chick caused the death of the chick, provided the donor of the implanted tissue was near the stage of hatching or had hatched. Neither implantation of nonmacerated tissue nor of macerated chick kidney to the chorio-allantoic membrane of the duck had this effect. Other kinds of macerated duck tissue have apparently not yet been tested. Sandstrom believes that the death of the chick in this experiment was caused by an agglutination of erythrocytes within the blood vessels. However, it is not certain from his report whether the occlusion of the vessels was due to a pure agglutination process or whether coagulation processes had been involved in this effect; it seems possible that it was due

Chapter 16

The Relations Between Processes of Immunity and Individuality Differentials in Transplantation

THE OBSERVATIONS made by surgeons and by experimental biologists, which showed that in man and in higher animals autotransplantation succeeds much better than homoiotransplantation of various organs and tissues and that heterotransplantation never succeeds, gave rise to various interpretations as to the cause of these differences. In tracing the development of these interpretations it is interesting to note that they depend largely on two factors. In the first place, the discoveries made and the systems of thought built up in different fields of science are seen to be related to particular problems certain analogies are observed or are assumed to exist between two different series of investigations and the conclusions of the one are applied, with some modifications, to the other. Secondly, new experiments are carried out in order to analyze a problem by a direct approach, but here, also, the interpretation may be influenced by analogies with conclusions arrived at in the related science. These two factors are clearly discernible in the search for an answer to the question as to why homoiogenous transplantations do not as a rule succeed. Towards the end of the last and in the beginning of this century, the thoughts of pathologists, in their analysis of transplantations of organs and tissues in higher animals, were influenced by the investigations of experimental biologists, who grafted tissues in lower animals and plants and who found polarity in the structure of the organisms to be a factor in transplantation, and who also observed that the character of the tissues adjoining each other in host and transplant was of great significance in determining the compatibility of grafts and hosts, and it was mainly for the purpose of discovering polarity and other related factors as determiners of normal structures that biologists carried out experiments in grafting. Such an influence is noticeable in the work of the pathologist Marchand on transplantation in higher animals and in man, in the writings of Lubarsch; and also to some extent, in those of Schoene. Then in the beginning of this century, the differences between the results of autogenous and homoiogenous transplantations of tissues and tumors in higher vertebrates were interpreted as due to the various degrees of compatibility or incompatibility between the chemical composition of the bodyfluids of the host and of the transplanted tissues. This interpretation was suggested by us, and the importance of the biochemical constitution of host and graft was also emphasized by Borst, who believed that inadequate biological systems may cause atrophy and loss of function of transplanted organs; Borst considered, in addition, the effect of cytolysins and anaphylaxis, assuming that such factors

examination, a reaction of the host may be demonstrated, which otherwise would not have become manifest. In these respects, the primary blood group antigens and individuality differentials differ from each other. (3) While individuality differentials have been found in all vertebrates so far studied, and at least as far down in the phylogenetic series as the anuran amphibia, there seem to be great variations among different species and classes in regard to the presence, number and character of the blood groups. (4) While the reactions against individuality differentials manifest very fine gradations in response in accordance with the genetic relationship between host and transplant, the blood group reactions are sharply defined into essentially two classes, namely, those of compatible or incompatible individuals.

In certain respects the blood group factors of primates have an intermediate position between the organismal and tissue differentials, having certain features in common with both. As far as the organismal differentials are concerned, we shall discuss later their phylogenetic evolution; it would be of interest to trace in a similar manner also the evolution of the blood group antigens. Such a study might help to clear up still further the relationship between the blood group factors and the organismal or individuality differentials.

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may explain, as well, the occasional lack of success in autogenous transplantations, while we held that the controlling factors are identical in hosts and in autogenous grafts.

It was at this point in the history of transplantations that the viewpoints developed in immunology and serology began to be applied. It was believed, with some justification, that these differences in the chemical constitution of host and transplant might be due to differences in the structure of proteins or of a certain protein in host and transplant, and that after transplantation of a tissue into a different host, such a protein might give rise to antibodies, comparable to hemolysins or agglutinins. Furthermore, anaphylactic phenomena were used in explaining the destruction of the transplants in unfavorable hosts. While we stressed the concept that the primary incompatibility between bodyfluids and tissues of host and transplant, as such, may lead to toxic injury of the transplant, the large majority of investigators thought at that time that immune processes, taking place in the host against the graft, were the principal factors that produced the injury and destruction of the graft after homoiogenous and heterogenous transplantation, although it was considered possible that a primary toxicity of the bodyfluids might play a minor role. This point of view was presented especially by Schoene, a collaborator of Ehrlich, and there was some direct experimental evidence in favor of this interpretation. In the case of tumor transplantation it had been possible to demonstrate an active immunization of the host as the result of the growth and regression of a primary tumor and of various other conditions, and Russell went so far as to maintain that in every case the lack of success in transplantation of tumors as well as the regression of transplanted tumors was due to the development of an active immunity against the tumors. The reaction of the host towards the transplanted tumor was assumed to be the consequence of the development of immunity in the host and the period necessary for the appearance of a reaction should accordingly correspond to the time required for the production of an immune state. This view was accepted also by Tyzzer and Burgess, and by various other investigators, and Tyzzer applied this conception to the reaction on the part of the lymphocytes.

In the case of normal tissues, Schoene found it possible to immunize a rat actively against mouse organs; such an immunized rat reacted more rapidly against a subsequently transplanted piece of mouse skin. It was more difficult to elicit immunity against homoiogenous skin. But Schoene succeeded, by preliminary treatments with embryo skin, kidney or liver of rabbit, in immunizing another rabbit against homoiogenous tissues, so that, 24 days after grafting, a homoiogenous skin transplant was more rapidly destroyed while autogenous skin was not affected. The more closely donor and host were related, the more difficult it was to produce such an immunity. Accordingly, skin grafts between brothers and sisters were more successful than those between distant members of the species; yet Schoene did not recognize the significance of genetic factors in transplantation. Also, the observation that skin grafts could apparently heal in for two or three weeks and that only then were they cast off, was interpreted as indicating that a certain time had to elapse before

the active immunity could establish itself. There were, in addition, the experiments of Fichera, who showed that it was possible to immunize rats against grafts of rat embryo by successive transplantations of the tissues of rat embryos, and those of Peyton Rous, who obtained similar results with mouse embryos. Likewise, repeated transplantation of adult skin seemed to lead to a more active destruction of the last transplant. There may be cited, besides, the finding of von Dungern, that rabbits could be immunized against the tracheal epithelium of cattle, which was then more rapidly destroyed by the bodyfluids of rabbits. Subsequent investigators, as for instance Lehmann and Tammann, as well as Fischer, assumed that the development of an active immunity was the cause of the lack of success in homoiotransplantation. The former conceded, however, that with a heterogenous serum a primary toxicity may play a certain role, but that this would be of slight importance in homoiogenous transplantation.

In addition to the active immunity, some other factors were thought to cause the destruction of homoiogenous transplants. Ehrlich had observed that growth of a first tumor could prevent the growth of a second tumor in certain cases, and he also noted that transplantation of a tumor piece into a pregnant animal did not succeed well; he interpreted these effects as being due to a competition for specific foodstuffs, in which an established tumor or a growing embryo had the advantage over a recently transplanted tumor, which thus, suffered from athrepsia. To this factor, starvation, Ehrlich attributed also the slow death which a mouse tumor underwent when it was transplanted to a rat. In a similar way Schoene explained the fact that homoio- or heterotransplanted skin could be successfully re-transplanted to the original donor after it had been in the new host for three days, whereas, after a period of four days the injury of the skin graft was so severe that a successful retransplantation was no longer possible.

Among still other factors considered as responsible for the death of homoiotransplanted tissues, may be mentioned lack of function. The importance of this factor was especially indicated by an experiment of Jores, which showed that electric stimulation exerts a beneficial effect on transplants of striated muscle. Also, deficient nourishment and older age of the host resulted in less successful transplantation, as did also, according to Ribbert, differences in the composition of the inorganic salts of host and donor. Schoene accordingly believed that factors, such as favorable conditions for function and nourishment in the host, may make possible a successful homoiotransplantation. However, less significance was attached to these factors by later investigators, who, as stated previously, stressed above all the importance of an active immunization of the host against the transplant as the cause of the destruction of homoiogenous and heterogenous grafts. That so little importance was attributed to the primary incompatibility between the bodyfluids and tissues of host and transplant seems to have been due largely to two factors. In the first place, the reactions taking place between the hemolysins and bacteriolysins, the agglutinins and precipitins, which were considered as types of primary toxins, and the cells providing the antigens occur very rapidly and inasmuch as so

rapid an injurious effect was not observed in the case of transplanted tissues, the importance of such toxins in transplantation was ruled out. The possibility that in homoiogenous transplantation we may have to deal with primary toxins of a different kind, acting less acutely but rather slowly in a gradually cumulative manner, was not sufficiently taken into account. In the second place the conclusion of these investigators, were based to a large extent, on naked-eye observations of skin grafts and not on the microscopical examination of successive stages in the process of transplantation. The latter would have revealed the fact that a reaction of the host may set in at a much earlier stage than would have been otherwise expected; the specific cellular reactions of the host could begin as soon as the transplant had sufficiently recovered from the injuries inflicted by the operation and by the transfer into a strange soil.

The cellular reactions of the host against the transplant were likewise attributed to immune processes which develop in the host. In the case of tumor transplantations, cellular infiltrations were observed by Da Fano in various places in the host; these cells were lymphocytes, plasma cells and macrophages, and they were interpreted by Da Fano as indicators of, as well as instruments in the production of an active immunity against the transplanted tumor, and also the numerous observations of Murphy as to the significance of lymphocytes around transplanted tumors were in harmony with this view. On the other hand, we interpreted cellular infiltrations, principally of lymphocytes, around and in the homoiogenous transplants as being caused by the difference in the individuality differentials of host and transplants, these cells being attracted by the strange individuality differentials of the grafted tissue; their presence indicates the existence of variable degrees of incompatibility between tissues and the action of a mild rather than that of a severe, acutely acting toxin.

We tested, partly in collaboration with Cora Hesselberg, the effect of a first transplant on a second transplant in several series of experiments in guinea pigs and rats of different ages. The first transplants remained in the host for periods varying from two to twelve days. Control experiments were made for the first as well as for the second transplants. Single instead of double transplants served as controls in some instances; in others, a piece of paraffin was inserted instead of a first transplant. In general, it may be stated that no definite effect of the first on the second transplant was noticeable in these experiments, the condition of both transplants varying within the same range as those in the controls. If we assume that it was the development of an immune state in the host which caused the reactions against the graft, we should have expected that about eight to twelve days after transplantation of the first pieces an immunity was established and that, accordingly, the lymphocytes were ready to attack the strange homoiogenous tissue. A definite lymphocytic reaction should therefore have developed around a second transplant within the first five days after transplantation. This, however, was in no case observed; instead, the reaction occurred at about the usual time and as usual there were considerable variations in the strength of the reactions against the

transplants and in the preservation of the latter; under these circumstances it was very difficult to recognize the possible presence of slight effects of a first on a second graft. However, as a rule, in accordance with expectations, the reactions against the first and older graft were more severe, on the average, than the reactions against the second graft. Furthermore, thyroid gland and cartilage and fat tissue, which were the tissues transplanted in the majority of cases, again behaved in a corresponding manner in the individual experiments. It was likewise noticeable that the same host reacted with different degrees of severity against homoiogenous transplants which had originated in different donors, and different hosts seemed to differ in the severity of the reactions against the same donor. There was in a number of instances, in the same host, a correlation between the fate of the first transplant examined 33 days after transplantation and of a second transplant examined after 12 days. When the first transplant was relatively well preserved, or when the reaction against it was severe, also when there was much lymphocytic infiltration in the first transplant, similar conditions were found in the second transplant. This indicates that the degree or reactivity of the host against homoiogenous differentials was one of the factors that determined the results of the transplantations. From these experiments we may therefore conclude that immune reactions in all probability are not of paramount importance in determining the reactions against homoiogenous transplants, but on the other hand, we cannot exclude the possibility that slight immunizing effects may be exerted by a first on a second homoiogenous transplant.

The primary factors determining the fate of transplants are, then, the differences in the individuality differentials of host and transplant, these differentials being preformed and giving rise to relatively slow and mild, but, in many instances, gradually accumulating primary reactions against the transplants. The reactions of the host against the transplant set in as soon as the latter has entered into organic connections with the host and as soon as the individuality differentials have had a chance to diffuse from the transplant into the host and here to set in motion a response on the part of the various tissues.

The investigations of M. S. Fleisher agree with our conclusions. He found that immunization of an animal by homoiogenous tissues did not in any definite way modify the course of the typical reaction against homoiogenous transplants. Conditions were different in the case of heterogenous grafts; here as a result of immunization there was an increased accumulation of polymorphonuclear leucocytes around the graft in the first few days following transplantation, as well as a delay in the ingrowth of fibroblasts into it, and furthermore, a reduction in the slight growth processes which may take place in a heterogenous host; but these differences between immunized and normal hosts were only transitory; they soon subsided. These experiments are furthermore in agreement with the view that heterogenous tissues are more efficient sources of antigens than are homoiogenous ones, because the former are more strange and are therefore more prone to initiate immune reactions. However, it seems

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enous and heterogenous tissue may induce immunity reactions against tumor as well as against normal tissue. (3) These secondary immune reactions also are relatively slight as compared with the primary reactions occurring as the direct result of the diffusion of the individuality differentials from a transplant into the circulation of the host. The primary individuality differentials are therefore responsible for the major portion of the reaction of the host against homoiogenous tissues; but secondary, immune reactions may also participate in this reaction, although only to a lesser extent.

These investigations as to the nature of the reactions against homoiogenous and heterogenous tissues suggested a second problem, one which was of practical importance and which therefore interested surgeons especially, because its solution might be an aid in the grafting of homoiogenous tissues in human beings. This problem concerned the possibility of improving the results of homoiotransplantations by experimental means. It was thought possible that in animals, through a preliminary treatment of the host with blood serum or plasma, or with tissue extract of the donor, the former might become desensitized to the effect of the homoiogenous tissues. These experiments were, however, unsuccessful. Likewise, the treatment of the transplants with similar substances from the host previous to the grafting did not cause an accommodation of the homoiogenous tissue of the donor to the conditions found in the host. Nor was it possible by means of parabiosis between donor and host to prolong noticeably the life of the homoiogenous tissue, although under these conditions the graft was supplied with some of the blood to which it was adapted. The observation of Murphy and his collaborators, that by the application of Roentgen rays to the host, and by other means which tended to reduce the number of available lymphocytes, a more favorable outcome in the transplantation of homoiogenous tumors could be obtained, induced surgeons to apply the same methods to the homoiotransplantation of normal tissues, but no real improvement was attained.

In some of the papers of earlier investigators, in which the question as to the causes of the usual failure of homoiogenous transplantations was discussed, frequent reference is made to a state of anaphylaxis, resulting from grafting of tissues, as one of the principal factors involved in this process. However, a statement as to the nature of such an anaphylactic state and its distinction from a condition of immunity against the transplant in the host is not usually made. In order to test this assumption regarding the presence of anaphylaxis in transplantation, experiments were made by us in which conditions were favorable for the development of a state of sensitization and thus also for a subsequent state of anaphylaxis in the transplant. We sensitized guinea pigs by injecting them with horse serum and afterwards transplanted pieces of uterus, thyroid or ovaries of the sensitized animal into other guinea pigs, which had not previously been injected with horse serum but which were injected sometime after they had received the transplants. In other experiments we transplanted corresponding tissues from non-injected guinea pigs into animals which received an injection of

that also in the case of heterogenous tissues the primary reactions due to the preformed organismal differentials are the more essential factors on which depend the fate of the transplants.

The data so far discussed suggest, then, the interpretation that the reactions of the host against both homoiogenous and heterogenous tissues are due largely to the direct, primary action of the individuality differentials given off by the transplants, but that, secondarily, these differentials may also act as antigens and induce the formation of immune substances, which then may secondarily intensify the strength of the reactions. However, it is apparent also that by means of successive transplantations it is difficult to decide the question as to how far such immune processes participate in these reactions. This method suffers from the disadvantages that each transplant can be examined only at the end of the experiment and that it is necessary to study the tissue in stained section. The examination of the white blood cells as a method for the analysis of the individuality differentials obviates these disadvantages, although in certain respects the study of the local reactions around the transplanted tissues is preferable. By determining the effects of two successive transplantations on the white blood cells circulating in the peripheral blood, Blumenthal demonstrated the existence of immune reactions also against transplanted tissues. In these experiments, the interval between the two transplantations was 10 or 21 days. In the case of successive transplantations of homoiogenous tissues, the second transplant called forth an accelerated increase in the lymphocytes in the blood. The maximum number of these cells appeared from two to four days earlier than after transplantation, but the maximum number of cells counted after the second transplantation was not so high as after the first transplantation in the large majority of cases. This effect of a second transplantation was noted only if both the first and second transplant were homoiogenous, but not if one of the two grafts was of a heterogenous nature.

If successive heterogenous transplantations were made, the maximum in the increase of polymorphonuclear leucocytes, as well as of lymphocytes, which took place as the second phase of the reaction of the host against the transplant, was accelerated to about the same extent as after successful homoiogenous transplantations. But, again, the maximum number of cells found in the blood after the second transplantation was lower than that found after the first transplantation. In order to obtain this effect, both the first and second transplant had to be heterogenous; again a combination of a heterogenous and a homoiogenous graft did not produce this effect. The immunizing effect of homoiogenous and heterogenous tissue on the lymphocytes and polymorphonuclear leucocytes in the circulating blood is therefore a specific one. Immunization against the growth of transplanted homoiogenous and heterogenous pieces of tumors may, to a certain degree, be accomplished by a first transplantation of normal tissues, and here, also, both the first transplant of normal tissue and the second transplant of tumor must be either homoiogenous or heterogenous. We may conclude from these experiments: (1) that the reactions against homoiogenous and heterogenous tissues differ not only quantitatively but also in kind, and (2) that also normal homoiog-

In accordance with the relatively slight effectiveness of this method, if applied to homoiogenous skin grafts, it was found that splenectomy, which causes a partial elimination of the reticulo-endothelial system, was ineffective as far as improvement in the results of homoiogenous transplantation was concerned. Also, in our experiments splenectomy did not weaken noticeably the reactions of the host against homoiogenous transplants of thyroid and other tissues. There still remains the method used by Rhoda Erdmann for this purpose in amphibians, in experiments which we shall discuss subsequently. In general, as was to be expected on theoretical grounds, we may then conclude that it has not been possible to change experimentally the individuality differentials of tissues, although it may be possible to influence the reactions of the host against strange differentials by certain experimental procedures. As to the reason why the local reaction around transplanted normal tissues does not reveal definite processes of immunity, under conditions in which such effects can be demonstrated in the case of transplanted embryonal or cancerous tissues, this may be due to the fact that both pieces of embryonal and of tumor tissue, as a rule, grow much more rapidly after transplantation and metabolize more actively than normal tissues and that in all probability the former correspondingly give off larger amounts of effective antigen.

horse serum during the period following the transplantation, and in a third type of experiments, injections were given to the host, both prior to and following transplantation. It was conceivable that the transplanted tissues in some of these instances had been sensitized to horse serum and that a second injection of horse serum would cause an anaphylactic reaction in the transplant, which would alter the state of preservation and the lymphocytic, connective-tissue and blood-vessel reaction of the host towards the transplant. However, this was not the case; the reactions of the host tissues against the graft were not essentially altered by these procedures. Only in a few cases, in which the general health of the guinea pig serving as host had been affected by the injection of horse serum, was a definite effect on the transplant noticeable, but it is not probable that in the last named experiments we had to deal with a specific condition of anaphylaxis in the transplant. As far as our experiments make possible a decision in this respect, it may then be concluded that a state of true anaphylaxis is not one of the factors which underlies the reaction of the host against homoiogenous transplants. As to a possible improvement in the results of homoiogenous transplantation, only one method appeared to be able to exert such an effect, and this was the inactivation of the reticulo-endothelial system by means of injection of trypan blue into the host previous to the transplantation. In the experiments of Lehmann and Tammann, such a procedure seemed to prolong the life of the transplanted piece of skin to a moderate degree. In 16 out of 28 mice so treated, the skin grafts were better preserved after having been in the hosts for four weeks than in controls; also, the staining of the skin to be transplanted by trypan blue seemed to protect it to some extent against the antagonistic processes which as a rule take place in the host after transplantation. Trypan blue was effective presumably because, temporarily, it diminished the cellular response in organs where the lymphocytes are activated by the homoiogenous tissue, and also it may, perhaps, have neutralized primary antagonistic constituents of the bodyfluids which otherwise would have acted on the strange grafts; furthermore, it is possible that the individuality differentials of transplanted skin infiltrated with this dye are rendered ineffective for a certain period. But on the whole, these effects are weak and temporary. The use of trypan blue proved to be ineffective in similar experiments in rabbits, and Villata also obtained negative results with this method when applied to transplantation of bones and joints in rabbits. In similar experiments by Blumenthal with guinea pigs, into which trypan blue was injected, the rise in the number of white blood cells otherwise caused by the implantation of homoiogenous and heterogenous tissues was prevented; but he noticed that when tryan blue exerted such an effect, the transplant was surrounded by a peripheral ring in which this dye was deposited. He concluded, therefore, that it must be left undecided whether the deposit of the dye in the periphery of the transplant inhibited the diffusion of the organismal differentials into the host, or whether the trypan blue inhibited the reaction on the part of the leucocytes through a blockade of the reticulo-endothelial system.

skin junctions, but in some cases there may be, in addition, connections through large omental blood vessels. To the peritoneal-skin union there is usually added, in parabiosis, a union by skin flaps, which increases the size of the area of vascular connection between the two animals. Characteristic of parabiosis is, then, the combined action of two systems of bodyfluids and of two kinds of individuality differentials in the same individual; however, there is a great quantitative predominance of the animal's own constituents over the strange ones carried to him from his partner. There are some related parabiotic states which differ in various respects from the typical parabiosis just described. Thus it is possible to transplant skin and certain other organs to a strange individual by means of a pedicle containing blood vessels, which keeps the transplant united with the original donor; it then received blood from the latter, while at the same time it receives bodyfluids from the new host, and is accessible, to a limited extent, to the action of the host cells. The union between child and mother in the uterus, by means of the placenta, may also be considered as a modified state of parabiosis, in which both organisms lead largely an independent life and in which both carry on their own metabolism, but in which to a certain degree an exchange of substances may take place through the placenta. In a still wider sense, the condition of symbiosis, or of parasitism, may be considered a state of parabiosis, in which host and symbiont or parasite carry on essentially their own metabolism and function, each in its own peculiar manner, but in which substances may be exchanged between the two organisms. We are concerned here with these various conditions only in so far as the action of individuality differentials on strange tissues, organs, or whole individuals comes into play.

Transplantation by blood vessel anastomosis. Hoepfner (1903) first carried out the retransplantation of an amputated leg in a dog by uniting blood vessels, muscles and skin. The dog died as a result of an accident after eleven days; the vessels were found free from thrombosis; skin and muscles at the place of union between host and transplant showed satisfactory healing and there was a tendency of the bones to unite. Several years later, Carrel and Guthrie, Carrel, as well as Lexer and Giani, made similar experiments; but Carrel improved the method of blood vessel anastomosis and in addition extended transplantation by this method to kidney, thyroid, adrenal gland and ovaries. He believed that not only autotransplantation but also homoiotransplantation of arteries may succeed. After a few months, the microscopical structure of the transplanted vessels was almost, although not entirely, normal. Both Carrel and Guthrie found that even after heterotransplantation of carotid from dog to cat, the vessels were normal after more than one year. When rabbit vessels were grafted to a dog, the function of the artery was maintained for a long time, but only the connective tissue constituents of the arteries remained preserved. Not only autotransplantation, but also homoiotransplantation, of kidney, thyroid, adrenal and ovary succeeded. The kidney functioned, although hydronephrosis and interstitial nephritis were observed in some cases in the transplanted organ. Likewise,

Chapter 17

The Significance of the Individuality Differentials in Transplantation by Means of Blood Vessel Anastomosis and in Parabiotic States

So far, the interaction of the individuality differentials of host and transplant and the effect of various factors on this interaction have been considered under conditions of a complete primary separation of the grafts from the surrounding tissues, only later a union taking place between the transplant and the tissues of the host. Such a transplant lives under unfavorable conditions of nourishment during the first few days following the transplantation and the central parts of the graft, which suffer most from insufficient nourishment, undergo necrosis. This disadvantage is eliminated if directly after separation of an organ or of a part of an animal, the large blood vessels, and perhaps even the nerves, of the transplant are connected with the corresponding structures of the host at the site of transplantation. Thus the blood of the host is carried at once to all parts of the transplant, which does not then suffer from lack of nourishment and the central necrosis is prevented. The homoiogenous or heterogenous individuality differentials act, therefore, in this case, on tissues which are well provided with food and should be better able to resist the unfavorable action of the host. Moreover, the individuality differential substances produced in the transplant, instead of diffusing slowly into the adjoining area, have a chance to be carried directly by vessels into the general circulation of the host, where they are much diluted. It may therefore be expected that the local reaction around the transplant, which is so prominent a feature in the ordinary kind of transplantation, is lacking around transplants which are joined to the host by means of blood vessels.

In parabiosis—a method of transplantation which was first conceived and applied by Paul Bert, but was technically developed in its present form by Sauerbruch and Heyde—two individuals, usually belonging to the same species, but sometimes also to different species, are united by establishing by means of incisions and sutures a connection between the peritoneal cavities as well as between the skins of the two animals. In parabiosis, two individuals are therefore incompletely joined together; essentially, both partners continue their individual metabolism and functions of organs and live their own life, but at the same time some substances, including individuality differentials, have a chance to pass continuously—although at a slow rate—from one partner to the other; this takes place mainly by way of capillary anastomoses, connections which gradually develop at the site of the peritoneal-

rule. Difficulties arise in the latter two, at the place of junction between the vessels of donor and host because of tissue and bodyfluid incompatibility, and thrombi often develop, but even without such thrombi, other than autogenous tissues are injured and ultimately succumb. This applies also to homoio- and heterotransplanted organs, which apparently regress relatively soon after transplantation. There is some difference of opinion as to the length of time during which transplanted pieces of arteries can remain alive, but this seems to be a point of less importance. Certain connective tissue structures may live presumably longer than the more sensitive constituents of these transplants.

From a theoretical point of view, these transplantations by blood vessel anastomosis are of special interest, because they make possible a separation of the effects of the bodyfluids on transplanted tissues from those of the host cells. The latter effects are, in all probability, as far as the incomplete reports on the results of microscopical examination make an evaluation of this factor possible, either entirely lacking or very slight under the given conditions of experimentation. It is essentially the injurious action of bodyfluids, carrying disharmonious individuality and species differentials from the host to the grafted tissues, which accomplishes the destruction of homoiogenous and heterogenous tissues and organs. However, those investigators who express an opinion as to the cause of the lack of success of homoio- and heterotransplantations, as, for instance, Borst and Lexer, stress two specific factors. In the first place, it is assumed that the homoiogenous and heterogenous transplants cannot make use of the specific foodstuffs of the host and, therefore, after using up their own reserve material, they starve, and that secondly, cytolysins or related immune substances develop as a result of processes of immunization taking place in the host. Even Borst, who emphasizes the biochemical differences between host and transplant as the primary cause of the state of athrepsia and immunization, has in mind specificities inherent in various organs and tissues, which require not only the adequate nourishment but also the normal function of these structures in order to overcome adverse conditions. Biochemical differences, in the way he applies this term, refer largely to or include the tissue differentials. This concept differs therefore from that which holds the differences in individuality and organismal differentials as primarily responsible for the changes characteristic of the various types of transplantation.

Transplantation by pedicled flaps. It is especially in skin transplantation that pedicled flaps are used. This method of grafting tissue resembles transplantation by blood vessel anastomosis, in so far as the transplant has, from the beginning, a satisfactory blood supply, reaching it in this instance through the vessels of the pedicle, which originate in the donor of the skin graft. Secondarily, the skin flap makes connection with the vessels of the host, from whom it then also receives blood. Thus its own blood vessels carry to the transplant substances bearing autogenous individuality differentials, while the blood vessels coming from the host carry to it substances bearing strange individuality differentials. The pedicle-flap mode of transplantation, therefore, differs from that by blood vessel anastomosis, which has just been

homoiotransplantation of a leg from one fox terrier to another was successful. The animal lived 22 days after the operation, all the tissues had healed, there was no ulceration, and even regeneration had taken place when a toe in the grafted limb was injured. A callus united the bone ends. In later experiments, Carrel observed, in a homoiotransplanted kidney, secretion of urine for 8 days, but the animal died after ten days. After heterotransplantation of kidney, the transplant was absorbed a few weeks later. Lexer did not find it possible to keep a transplanted leg in a dog alive for longer than three weeks, and thrombi were found to develop in the transplanted tissues. Giani however was more successful and a leg autotransplanted by him lived for three months; there was good union but no active motion.

However, the results of careful microscopical studies made by Borst and Enderlen on transplanted blood vessels showed that only autogenous transplants survive for any length of time; homoiogenous and also heterogenous transplants gradually die and are replaced by the tissues growing into the transplant from the adjoining host tissues and wandering cells of the host may accumulate at the point of union. In this place thrombi form more frequently after homoio-, and especially after heterotransplantation, than after autotransplantation. Autotransplantation of thyroid and kidney by means of blood vessel anastomosis may succeed; but after homoiotransplantation, hemorrhagic infarction, necrosis, or atrophy and fibrosis of the grafted organ occur. Likewise Williamson found that the homoiotransplanted kidney functioned only for a few days, while autotransplantation was successful, except that atresia of the ureter could cause hydronephrosis and infection of the graft. In case of syngenesiotransplantation of kidney within the same litter of dogs, kidney function was maintained for 26 days. As to the length of time during which homoiotransplantation of blood vessels may succeed, Ingbrigtsen observed, in cats, that the carotids may remain alive for three months; among 14 experiments, 8 satisfactory results were obtained. There was no thrombosis in these latter cases, but this did occur in the other six. Elastic fibers of media were normal, likewise muscle cells were well preserved, while intima and adventitia were thickened.

The interpretation of these investigations, as far as they are of interest in the analysis of the individuality differentials, suffers from a lack of distinction, in the reports of the authors, between strict homoiogenous and syngenesious (brother-sister) relationship in many of the experiments. Also, too great a reliance was placed on a mere macroscopic examination of the transplant, while careful microscopic studies of successive stages in such transplantations were omitted. Furthermore, these experiments were made largely from the viewpoint of the surgeon, who is interested in the possibility of using such methods of transplantation in patients. Notwithstanding the difficulties involved in a correct interpretation of the results of these investigations, and of other similar ones, which need not be discussed, it may be concluded that there is a marked difference between the fate of autogenous, homoiogenous and heterogenous parabiotic transplants. The former may live indefinitely if unfavorable conditions of a more or less accidental kind can be avoided, whereas, the two other types of transplants in all probability die as a

rule. Difficulties arise in the latter two, at the place of junction between the vessels of donor and host because of tissue and bodyfluid incompatibility, and thrombi often develop, but even without such thrombi, other than autogenous tissues are injured and ultimately succumb. This applies also to homoio- and heterotransplanted organs, which apparently regress relatively soon after transplantation. There is some difference of opinion as to the length of time during which transplanted pieces of arteries can remain alive, but this seems to be a point of less importance. Certain connective tissue structures may live presumably longer than the more sensitive constituents of these transplants.

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discussed, in that in the latter case the transplant is supplied exclusively with the blood of the host. However, in addition experiments have been carried out in which, first, two animals were united by parabiosis and then various organs were transplanted by blood vessel anastomosis from one partner to the other. Likewise, skin has been quite commonly transplanted by means of pedicled flaps to a parabiotic partner. Indeed, the transplantation of pedicled skin flaps from one animal to another represents a rudimentary parabiosis. If a pedicled skin flap is transplanted to another region in the same individual, and the pedicle is cut after healing has taken place, the transplant may continue to live during the lifetime of the person or animal. In the case of homoiogenous transplantation the skin graft remains preserved usually as long as the skin is united by the pedicle with the circulation of the donor and the transplanted tissue receives, through the blood, substances carrying its own individuality differentials. During that period it is sufficiently under the influence of autogenous substances to be able to resist the action of homoiotoxins which are active at the point of union. But if, after healing has taken place, the pedicle is cut, the transplant is fully exposed to the antagonistic reaction of the host cells, as well as to the homoiotoxins of the bodyfluids of the host, and its fate does not differ from that of the ordinary homoiogenous transplant, the advantage gained by the flap method being merely temporary. It seems that, as in skin transplantation by the ordinary method, so also by the pedicle-flap method, after the pedicle is cut, the transplant may, at least in some cases, survive for a longer time, perhaps even permanently, provided the individuality differentials of host and graft are relatively harmonious, which may be expected especially if syngenesiotransplantations are carried out. Thus Lexer succeeded in keeping alive for eight weeks a skin-flap transplant from daughter to father. In this case, *the father received daily injections of blood serum from the* donor; when these injections were interrupted, the skin flap no longer remained preserved but was cast off by the host. Lexer attributed this result to the favorable action of the injections of donor serum, supplying suitable foodstuffs for the transplant; but later experiments in animals by Lexer and Keysser showed that such serum injections do not exert a beneficial effect on the transplant and it may be assumed that the near relationship of the individuality differentials of transplant and host was responsible for the favorable results obtained.

Transplants of organs by blood vessel pedicles to a parabiotic partner do not behave differently from corresponding pedicled skin transplants; the grafted organs undergo the fate of ordinary homoiogenous transplants and die after the pedicle has been cut. We see, then, that it is essentially the relationship of the individuality differentials which determines the outcome in these transplantations, as it does in those of the ordinary type. However, it seems that in man, not only strange individuality differentials but also blood group antigens (A) may pass from one partner through the vessels connecting the skin flap to the other partner, causing in the latter the production of antibodies and leading here to the destruction of the blood corpuscles possessing the antigen A. This occurrence was observed by Lauer.

But it is doubtful whether such an occurrence would take place in rodents, in which experiments of this kind are carried out most frequently; here, apparently, the actions of the individuality differentials greatly predominate over blood group antigens, if the latter play any significant role at all in these animals. The same considerations would apply also as far as the interaction of the partners in typical parabiosis is concerned; here, too, it is very doubtful whether in experiments in organisms other than man and monkeys, blood group antigens would be of any importance. But in the latter, they may be effective, and in man also the stage of pregnancy may be of importance. In women it has been observed that especially the antigen Rh may pass from the fetus to the mother, causing the production of antibodies which then may lead to changes in the fetus (Wiener, P. Levine).

Parabiosis and individuality differentials. Parabiosis is an extension of the method of transplantation by pedicle flaps, in which, in addition, union usually takes place between two individual organisms by the joining together of small areas of the peritoneal wall, and, in some cases, also of the intestines. However, it is a method devised primarily for the purpose of joining together or transplanting on each other, two organisms which are able to live independently, and which, in certain respects, continue to live independently even after the union has been accomplished. Each organism takes its own food and maintains its own metabolism; each is united with its partner mainly by means of capillary anastomoses, which gradually increase up to about two weeks after operation, after which time there may be again a diminution in the number of anastomosing capillaries owing to the pressure exerted by the developing scar at the site of junction. It has been found that the connection by lymph vessels is richer than that by blood capillaries; occasionally, though, there may be an additional connection by large vessels in the omentum. The partners, corresponding to host and transplant, continue therefore to be perfused largely by their own blood supply; but, at the same time a not very intense, but continuous, inflow of strange body fluids occurs, thus transferring to one partner products of the intermediary metabolism from the other partner, and above all, transferring also substances carrying the individuality differentials of the other partner.

Locally, at the point of union, at first a large amount of granulation tissue develops, consisting largely of fiibroblasts but containing also various kinds of leucocytes; it is uncertain whether this abundant tissue formation represents merely the sum of that which would normally be furnished by each partner in the course of wound healing, or whether in addition the influence of substances strange to each partner exerts a special stimulation on the granulation tissue. In contrast to the blood vessels of the two organisms which communicate with each other and may grow from one animal into the territory of the other, no spontaneous ingrowth of peripheral nerves takes place, the nerves of each organism remaining separate. This is probably the reason why diseases like rabies or tetanus, which are propagated mainly by way of peripheral nerves, do not in parabiosis progress spontaneously from one partner to the other. However, as Morpurgo has shown, it is possible to establish experimentally a union of a nerve of one partner with a nerve of

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Parabiosis and individuality differentials. Parabiosis is an extension of the method of transplantation by pedicle flaps, in which, in addition, union usually takes place between two individual organisms by the joining together of small areas of the peritoneal wall, and, in some cases, also of the intestines. However, it is a method devised primarily for the purpose of joining together or transplanting on each other, two organisms which are able to live independently, and which, in certain respects, continue to live independently even after the union has been accomplished. Each organism takes its own food and maintains its own metabolism; each is united with its partner mainly by means of capillary anastomoses, which gradually increase up to about two weeks after operation, after which time there may be again a diminution in the number of anastomosing capillaries owing to the pressure exerted by the developing scar at the site of junction. It has been found that the connection by lymph vessels is richer than that by blood capillaries; occasionally, though, there may be an additional connection by large vessels in the omentum. The partners, corresponding to host and transplant, continue therefore to be perfused largely by their own blood supply; but, at the same time a not very intense, but continuous, inflow of strange body fluids occurs, thus transferring to one partner products of the intermediary metabolism from the other partner, and above all, transferring also substances carrying the individuality differentials of the other partner.

Locally, at the point of union, at first a large amount of granulation tissue develops, consisting largely of fibroblasts but containing also various kinds of leucocytes; it is uncertain whether this abundant tissue formation represents merely the sum of that which would normally be furnished by each partner in the course of wound healing, or whether in addition the influence of substances strange to each partner exerts a special stimulation on the granulation tissue. In contrast to the blood vessels of the two organisms which communicate with each other and may grow from one animal into the territory of the other, no spontaneous ingrowth of peripheral nerves takes place, the nerves of each organism remaining separate. This is probably the reason why diseases like rabies or tetanus, which are propagated mainly by way of peripheral nerves, do not in parabiosis progress spontaneously from one partner to the other. However, as Morpurgo has shown, it is possible to establish experimentally a union of a nerve of one partner with a nerve of

the second partner, and then an ingrowth of a peripheral nerve into the second animal may take place and cross-reflexes between the two partners can be established. Apart from these data, the sequence of events taking place in the tissues adjoining the area of union has not yet been sufficiently examined microscopically to determine whether at this point accumulations of lymphocytes occur comparable to those which are found around and in the ordinary transplants of homoiogenous tissues. However, it is conceivable that the constant supply of its own bodyfluids to the tissue of each partner alters the reaction of the lymphocytes to the strange tissue of the parabiotic partner, which would otherwise be found.

The general changes occurring at the site of union may vary greatly in different cases. In some instances, the tissues of the two partners may not unite well and may separate soon after the operation; or a temporary union may occur, which is followed before very long by a separation; again, there may be a long-continued union, which still may ultimately be succeeded by a shrinking of the skin flaps and a separation. In the majority of experiments the union seems to last not longer than a few weeks, but Morpurgo succeeded in keeping a pair of parabiotic rats united for 9-12 months, and one case is known in which the union between two rats continued for as long as two years and five months (Goto). Rats are apparently best suited, and rabbits next best, for parabiotic union, and it seems that union between other species, such as cats or dogs, and perhaps even mice, can be established only with greater difficulty. The results of parabiosis are most favorable between young litter mates of about the same weight and sex; however, a union between rats not belonging to the same litter nor to the same sex also may succeed, but for technical reasons it is advisable to choose partners of about the same age. As stated, the establishment of a syngenesio-parabiosis, where the partners are brothers or sisters, is most favorable, although a homoiogenous parabiosis may also succeed, but on the average less readily. Still more difficult are unions between different races; but Irwin joined successfully different races of doves. A parabiotic union between individuals belonging to different species (heterogenous parabiosis) may, in rare instances, succeed for a very short time; thus Lambert joined a rat and a mouse for a maximum period of eight days, and even a union between a guinea pig and rat succeeded for as long as eight days. However, under these conditions no real wound healing occurred at the point of union and afterwards the skin flaps separated as in cases of disharmonious homoiogenous union.

In parabiosis, a small amount of blood, of peritoneal fluid, of lymph and interstitial fluid constantly passes from one partner into the other and here it is mixed with a much larger quantity of the animal's own fluids. There is reason, therefore, for assuming that the fluids of one animal reach, the cells of the strange organism, but only in very great dilution, and before they come into actual contact with the cells of the partner, they meet a current of fluid from the latter passing in the opposite direction, namely, from the cells towards the capillaries. The tissues thus constantly create their own individuality differentials, which move towards and mix with the very dilute strange differentials in such a way that a gradient of these differentials de-

velops. Hence the action of the bodyfluids of one partner on the other is very imperfect. We notice, accordingly, that substances which have a relatively low molecular weight and are not colloidal, such as KI, also products of the intermediary metabolism and certain toxins pass readily from one partner to the other. Other substances which have presumably larger molecules, with more colloidal properties, or substances such as some hormones which have strong affinities for organs in their own body and are here held back more readily, pass with greater difficulty and only under favorable conditions from one partner to the other. For instance, following extirpation of the kidneys of one partner, it is this partner which is primarily affected by the lack of these excretory organs; it suffers therefore from edema, also, in some cases, from hypertrophy of the left ventricle of the heart, and only under favorable circumstances can the compensatory hypertrophy which may occur in this animal save it from death by uremia (Morpurgo).

Similarly the interaction between the sex hormones takes place only imperfectly between the two partners, but castration of one partner may exert a stimulating effect on the sex organs of the other partner through the intermediation of the hypophysis (P. E. Smith, Matsuyama, Kellars, Martins, and others). If a pregnant and a non-pregnant rat are united, a growth may set in also in the mammary gland of the non-pregnant partner, but the reaction in the latter is weaker and delayed, indicating that some inhibition exists in the transmission of this effect. A passive immunity can be readily transferred from one partner to the other, but an active immunity can be induced in one animal through injections of the antigen into his partner only with greater difficulty; larger quantities of antigen must be used for this purpose. Likewise, if an animal susceptible to leukemia is united by means of parabiosis with a partner not susceptible to this disease, both individuals retain their specific degree of resistance to it, the leukemia being transmitted only to the susceptible individual; but transmission is more readily effected by injecting the leukemic blood cells into the susceptible animal directly than by injecting them into the non-susceptible partner. Also, in the case of a difference in susceptibility to transplanted tumors between two partners, as a rule each partner retains its specific state of susceptibility or lack of susceptibility; only in the case of Jensen rat sarcoma Zakrzewski observed that a Wistar rat, not susceptible to this tumor, can be made susceptible to it by the parabiotic union with a susceptible Warsaw rat. According to Simonnet and Pretresco, if a normally faster growing male rat and a more slowly growing female rat are joined in parabiosis, the growth rate of the partners is intermediate between the normal developmental rates of the two partners; an effect is thus transmitted in this case from each partner to the other.

As to the general effect of two parabiotic partners on each other, it is possible, in many instances, to distinguish two successive phases. As a rule there is at first a harmonious phase, in which both animals are relatively strong; this is followed sooner or later by a disharmonious phase, in which one of them becomes weak and atrophic, and eventually may die; but also the

surviving, dominating animal is usually not quite so well as it would have been in the free state. In this disharmonious phase the tone of the blood vessels is gradually lowered, especially in the weaker partner and towards the end of its life, consequently the stronger partner pumps a considerable part of its blood into the weaker one, which thus becomes hyperemic and polycythemic, while the dominating partner is made anemic. The diminished erythrocyte-destroying function of the spleen of the weaker animal has also been held responsible for the developing polycythemia. In this animal there are, in addition, atrophic changes in the inner organs and at last a fibrosis of the bone marrow may occur; in the stronger partner there occurs, on the contrary, a stimulation of the various lymphatic organs and of the bone marrow, with corresponding changes in the circulating cellular elements originating in the bone marrow. Nodules consisting of reticular-endothelial tissue may develop and accumulations of plasma cells may appear in the connective tissue. As a rule, the weaker hyperemic partner, whose heart is overloaded with blood, dies first, but occasionally the dominating though anemic partner may succumb earlier.

As to the cause of the disharmony which may develop, as early as in the second or third week after the union has been established, but much later in other cases, various suggestions have been made. Which of the two partners will be the less resistant one and will be ultimately suppressed seems usually to be determined by accidental factors, such as the presence of some inferiority, as for instance, lack of a kidney, at the time when parabiosis was established. The fact that disharmony develops at all has been attributed by some investigators to a state of undernourishment, on the assumption that the stronger partner deprives the weaker one of foodstuffs; however, the weaker partner often consumes a large amount of food and, also, the stronger partner may lose somewhat in weight. According to Hermannsdörfer, disharmony arises from the passing of intermediary metabolic products from one animal to the other. The increased toxicity of the urine in parabiotic animals, as indicated by the production of convulsions in other animals injected with such urine, has been considered as due to these substances. However, it is not probable that such metabolic products, which are present and are about the same in all normal individuals of the same species, are the essential cause of the disharmonious state.

Another view as to the underlying factor in this condition is held by a number of authors, who believe that a state of chronic anaphylaxis exists, due to the constitutional biochemical differences between the two partners, such differences arising from inherited differences in various organs. The dilatation of the vessels which is observed in the weaker partner is often cited as an important argument in favor of this view; but, this condition of the vascular system may be the result of general weakness rather than of specific anaphylaxis. Furthermore, if the two partners during the phase of disharmony are separated, a recovery of both may take place, and if subsequently, parabiosis is re-established, no sign of hypersensitiveness becomes noticeable. Moreover, the production of the well-known immune substances, such as hemolysins, agglutinins, or precipitins, can not usually be demon-

strated in parabiosis, although it seems that under special conditions hemolysins may develop. Thus Irwin found that in two different races of doves, in which no blood groups can be shown to exist, hemolysins may be produced; but in this case we have to deal not with a homoiogenous parabiosis, but with one approaching a heterogenous type. Also, Majeda states that he has observed the presence of hemolysins in a few cases. But the other investigators who have searched for hemolysins in parabiosis did not find them; or in those instances in which they were present, they had, in all probability, not been produced in response to the action of blood group antigens in the other partner, the existence of which could not be established, but they may have been due to the antigenic action of certain heterogenous or homoiogenous gene sets or their derivatives. The usual absence of the ordinary immune substances in parabiosis renders improbable also the existence of antibodies responsible for a state of anaphylaxis.

We suggested (1930) the possibility that substances which carry the individuality differentials are given off in small quantities by various organs of one partner and enter the circulation of the other, and that these may account for the gradually developing disharmony, the increasing atrophy of organs, and the weakness in the partner which was inferior from the beginning. Such substances may be expected to stimulate the lymphocytic and also the reticulo-endothelial system of the other partner, in accordance with the usual stimulation of lymphocytes in ordinary homoiogenous transplantation. These homoiogenous substances may thus conceivably function as very slowly acting toxins and secondarily may give origin also, although only feebly, to immune substances specifically directed against the individuality differentials of the partner.

In favor of this view, several facts may be cited: in the first place there is a parallelism between the genetic relationship of the parabionts and the rapidity with which disharmony is established and the intensity of the latter. As we have mentioned, the success of parabiosis essentially parallels the relationship of host and donor, in the same sense in which success of transplantation depends upon this factor; as in syngenesiotransplantation, especially in inbred strains, where the reaction against the transplant may become manifest only after a long period of latency, so also under favorable conditions of parabiosis the stage of disharmony may develop only after a long preceding harmonious state. Furthermore, the healing of the skin flap at the site of union of the parabionts behaves in a way which approximately corresponds to the genetic or pedigree relationship of the two partners; the healing-in succeeds the better the more compatible the two partners are with each other and the longer the harmonious phase lasts (Gohrbandt). During the disharmonious state it is especially the stronger partner which reacts more markedly against the skin flap. Of special interest in this respect are also the experiments of Majeda, who transplanted skin from one rat to another preceding and following the establishment of parabiosis. He found that those animals between which skin could readily be exchanged and remain preserved for some time were better adapted for parabiosis experiments, than were other animals in which skin transplants did not heal in a

satisfactory manner; if animals of the latter type were used, disharmony appeared earlier. Inasmuch as the conclusion is justified that the local reactions affecting transplanted skin are due to the action of strange individuality differentials, it seems also justifiable to conclude that, essentially, differences in individuality differentials are the cause of the disharmony which develops sooner or later in parabionts. Schoene, Majeda, and others, found furthermore that instead of improving the outcome of homoiogenous transplantation of skin, parabiosis, on the contrary, seemed to make the results more unfavorable, and similar observations were made also in autotransplantation. It is possible that the intensified injury, inflicted upon homoiogenous transplants as a result of parabiosis, is caused by an increase in the immune reactions against strange individuality differentials taking place under homoiogenous conditions. As to the injurious effect on autogenous grafts, we must consider the fact that a real autogenous transplantation in parabiotic animals is not possible, inasmuch as homoiogenous individuality differentials, even if much diluted, are continuously given off by the partner and must, to some extent, affect the condition of the autotransplant.

We may then conclude that in all probability strange individuality differentials are responsible for the injurious general as well as local reactions affecting partners in parabiosis, as for instance, for the damage inflicted on transplanted pieces of skin or of other organs, but that in addition to these primary direct actions of the strange individuality differentials, also immune reactions against these antigens may be active.

We have referred already to the analogy which exists between the state of pregnancy and that of parabiosis. However, pregnancy differs from true parabiosis in three respects: (1) In pregnancy, we have not to deal with the union of two formerly independent partners, able to sustain themselves in a free state, but with the development of an embryo and fetus inside the mother's organism; (2) the blood vessel connections between fetus and mother, by way of the placenta, are much more extensive than those existing between true parabiotic partners, and (3) the embryo and fetus receive essential foodstuffs from the mother. During pregnancy there is no indication that strange individuality differentials injure fetus or mother. Perhaps an enlargement of the lymphatic organs which may appear in the fetus might point to a late effect of strange individuality differentials, but this interpretation appears uncertain at present. On the other hand, it has been shown that in some rare cases antibodies may develop in the mother against blood group differentials present in the fetus, a point which has already been discussed. It is probable that during embryonic and fetal development an adaptation takes place against strange individuality differentials in both mother and fetus.

Also, parasitism may be considered as a condition resembling parabiosis. But this condition differs from true parabiosis in the very great inequality distinguishing host and parasite. The parasite lives at the expense of the host and is adapted in a peculiar manner to the host and to one or more of its organs. A further discussion of this relationship will be taken up in a later chapter.

Chapter 18

Modification of the Reaction of the Host Against Strange Individuality Differentials by Transplantation of Tissues Into the Allantois of Chick Embryos, Into the Brain, or Into the Anterior Chamber of the Eye

It has not been possible to prevent the injury or destruction of homoiogenous or heterogenous transplants by immunization of the host with blood or tissue extracts of the donor, or, conversely, by treating the donor with similar substances obtained from the prospective host. On the other hand, in a limited way, it was possible to protect the transplant against aggression by the host by inactivating the reticulo-endothelial system of the latter by means of injections of trypan blue. Previous to the last mentioned observations it had been shown that heterogenous mammalian tumors were able to grow in the chorio-allantois of the developing chick (Rous and Murphy), and subsequently the mechanism of this condition was analyzed by Murphy and his collaborators in a series of investigations. Murphy could show that after transplantation of a piece of spleen, and to some extent also of bone marrow, previous to or simultaneously with the transplantation of heterogenous tumor or of heterogenous embryonic tissue into the chorio-allantois, the transplants were destroyed in the same way as they were in adult hosts. The spleen tissue initiated the reactions of the embryo-host which, in the fully developed adult host, prevent the growth of heterogenous tumors and embryonal tissues, and correspondingly, the chicken embryo spontaneously became resistant to these strange transplants as soon as the embryonic development had reached the stage when the organs of defense could function. According to Murphy, these defense mechanisms consist largely in the activity of the lymphocytes, which call forth a state of immunity in the host. The immune processes thus produced acted not only against strange tumors, but they could also inhibit the growth of autogenous tumors and they were the same as those which protected the organism against pathogenic microorganisms, such as tubercle bacilli. All the means which injure or destroy the lymphocytes, weaken or remove the immune processes in the host and allow, therefore, tumors to grow or tuberculosis to spread, while those mechanisms which tend to stimulate the multiplication and activity of the lymphocyes, tend to intensify the immune processes and to protect the organism. By the application to the host of X-rays, dry heat, benzene, and certain other means which injure the lymphocytes, it is therefore possible to enhance the growth of malignant tumors in the organism.

As mentioned already, it is only at a certain stage in the embryonic development that organismal differentials are fully developed, and there is every reason to assume that the development of the mechanisms which are directed against strange organismal differentials takes place only subsequent to the complete formation of the organismal, and, in particular, of the individuality differentials. As we shall discuss more fully later, even in the very young guinea pig not long after birth the reactions against homoiogenous tissues are weaker than in older animals. The experiments of Murphy suggest very strongly that the spleen and the reticulo-endothelial system in general are the tissues which originate these mechanisms of defense, or at least aid in their development. We have furthermore seen that the lymphocytes are attracted by strange individuality differentials and that they may help in the destruction of homoiogenous tissues; but it is possible that also the individuality and species differentials, which are attached to certain substances in the bodyfluids and which exert a primary toxic effect on homoiogenous and heterogenous tissues, may develop with the aid of the reticulo-endothelial system. These considerations concerning the lack of individuality differentials and of the mechanisms of reaction against the latter in early embryos, would then explain why the chorio-allantois of the chick and, according to Murphy, also the chick embryo as such, do not oppose the preservation of heterogenous, actively-growing tissues, such as malignant tumors and embryonal tissues. According to Taylor, Thacker and Pennington, it seems that mammalian tumors grow very well also in the yolk sac of the chick embryo.

There are also, in adult animals, at least two sites where heterogenous tumor transplants may survive, namely, the brain and the anterior chamber of the eye. Shirai found that heterogenous tumors, which cannot be successfully transplanted elsewhere, may grow when transplanted into the brain. Murphy, who obtained similar results, observed that around such heterogenous transplants in the brain the usual lymphocytic reaction is absent; but, as in the case of transplantation into the allantois, a lymphocytic reaction can be called forth if simultaneously with the grafting of the tumor a piece of spleen is transplanted into the brain. However, when Harde transplanted homoiogenous tumors to the brain, no differences in results between the brain and the usual sites of transplantations were noted. Siebert compared with the reactions against tumor tissue, those against homoiogenous thyroid transplants in the guinea pig, the time of the examination of the graft varying between 20 and 120 days after transplantation. He found the amount of homoiogenous thyroid gland that was preserved in the brain less than that of autogenous transplants. Much fibrous-hyaline tissue developed in or around the homoiogenous graft. After 20 to 30 days, only a few small acini were preserved in the hyaline stroma. Lymphocytic infiltration appeared in the fibrous tissue invading the transplant and some scattered lymphocytes also surrounded the brain tissue, but on the whole, the lymphocytic infiltration was much less intense than after transplantation into subcutaneous pockets. The homoiogenous thyroid tissue remained longer preserved in the brain than it is usually in the subcutaneous

tissue, and it is possible that at a later period a moderate newformation of acini may have taken place in the graft in this site. We may then conclude that while in and around transplants of normal homoiogenous tissue the lymphocytic infiltration is diminished in the brain, the connective-tissue reaction is at least as marked as after subcutaneous transplantation. The mechanism which exerts a certain protective influence on homoiogenous grafts in the brain is therefore exactly the reverse of that which is active in young, as compared to older hosts. In young hosts the connective-tissue reaction is diminished, whereas the lymphocytic infiltration may be very marked; in the brain, the connective-tissue reaction may be quite pronounced, whereas the lymphocytic infiltration is weak. As to the difference in the mode of reaction in brain and in subcutaneous tissue of young animals, it seems improbable that this is caused by a lack of individuality differentials in the brain; it is more probable that the blood-brain barrier (L. Stern) prevents the homoio-toxin from reaching the brain in full strength, or at least diminishes its effectiveness. However, the possibility cannot be excluded that also other factors may be involved in this process.

Likewise in the anterior chamber of the eye transplantations, especially of organs with internal secretion, have been shown to succeed better than those made subcutaneously or intraperitoneally. Of considerable interest are the intra-ocular transplantations of testicle. As a rule, the testes of newborn animals, in particular those of rats, were used in these experiments, for instance, in the work of Pfeiffer, which we have already mentioned, and in the extensive investigations of G. D. Turner; furthermore, in rabbits (Bayer and Wense) conditions seem to be similar. The functioning of these transplants is greatly influenced by hormones; their presence exerts a stimulation favorable to spermatogenesis and their absence appears to cause degenerative changes in the more sensitive cells, so that only Sertoli cells survive. In all probability, stimulation by certain hormones is necessary to ensure the survival and function of the most differentiated cellular constituents of endocrine organs, particularly if these organs are under environmental conditions which are not quite adequate. Because the function of the sex organs may inhibit the formation of such stimulating hormones, these transplants may be more successful in castrated than in normal hosts, and in younger, sexually immature hosts than in older ones. However, it must be mentioned that the very favorable results of Turner were due to the fact that in addition to the very young age of the donors, donors and hosts were litter mates and the rats belonged to an inbred strain. These conditions eliminated to a great extent the action of unfavorable individuality differentials. But, it seems that after all, the individuality differentials of hosts and transplants were not entirely compatible in these experiments, and that under less favorable hormonal conditions these differences between the differentials could assert themselves and cause an invasion of the transplant by connective tissue and lymphocytes; at least this is the interpretation which might be given to some of Turner's experiments. That the organismal differentials do assert themselves also in the

As mentioned already, it is only at a certain stage in the embryonic development that organismal differentials are fully developed, and there is every reason to assume that the development of the mechanisms which are directed against strange organismal differentials takes place only subsequent to the complete formation of the organismal, and, in particular, of the individuality differentials. As we shall discuss more fully later, even in the very young guinea pig not long after birth the reactions against homoiogenous tissues are weaker than in older animals. The experiments of Murphy suggest very strongly that the spleen and the reticulo-endothelial system in general are the tissues which originate these mechanisms of defense, or at least aid in their development. We have furthermore seen that the lymphocytes are attracted by strange individuality differentials and that they may help in the destruction of homoiogenous tissues; but it is possible that also the individuality and species differentials, which are attached to certain substances in the bodyfluids and which exert a primary toxic effect on homoiogenous and heterogenous tissues, may develop with the aid of the reticulo-endothelial system. These considerations concerning the lack of individuality differentials and of the mechanisms of reaction against the latter in early embryos, would then explain why the chorio-allantois of the chick and, according to Murphy, also the chick embryo as such, do not oppose the preservation of heterogenous, actively-growing tissues, such as malignant tumors and embryonal tissues. According to Taylor, Thacker and Pennington, it seems that mammalian tumors grow very well also in the yolk sac of the chick embryo.

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heterogenous transplantation of thyroid from rat to guinea pig, living acinar tissue was found in two cases 10 days, and in two other cases, 18 days after transplantation; there was formation of hyaline connective tissue in and around these heterogenous transplants, and in some instances polymorphonuclear leucocytes collected around them. At dates later than 18 days, the transplants had disappeared. In this case, also, the results were somewhat better than in heterogenous transplantations into the subcutaneous tissue.

If we compare with these results, those obtained after intra-ocular transplantations of malignant tumors, the latter are much more striking. In a number of instances it has been possible to obtain active, continued growth in the anterior chamber of the eye, where none was found subcutaneously, and even heterogenous tumors, including human tumors, grew, in contrast to the subcutaneous grafts of this tissue. The difference between the growth of tumors obtained in these two sites is much greater than that of normal adult tissue. Results of this kind have been recorded by Smirnova, by Greene, by Greene and Saxton, by Appel, Saphir, Janota and Strauss, and by Cheever and Morgan; but Greene found that not all heterogenous tumors could be successfully transplanted into the eye; and the degree of success seemed to depend upon the original growth energy of the tumor used for transplantation. In some instances, also serial transplantations in the eye were successful. After retransplantation to the subcutaneous tissue, of tumors that had grown in the eye, the tumor cells died. The greater power of survival and growth of tumors in the anterior chamber of the eye as compared to that of ordinary tissues is in part probably due to the greater growth momentum inherent in tumors, which leads to a multiplication of the advantages offered by conditions in this site as compared with those present in the subcutaneous tissue. However it is possible that still another factor is active, namely, the diminution or lack of immune substances in this region. Tumors, as a result of their growth in hosts bearing different individuality differentials, as a rule seem to give rise to immune processes to a higher degree than do normal tissues, perhaps at least partly on account of their increase in mass, which takes place with relatively great rapidity, and tumors are very sensitive to the injurious action of such substances, especially during the first period following transplantation. In the case of ordinary tissues, as we have seen, the primary homoio- and heterotoxins are apparently very much more important in the determination of their fate after transplantation than are the immune substances, although the latter may play some role also; however, in the case of tumors there is evidence that though the primary homoio- and heterotoxins likewise help to determine the result after transplantation, the immune substances are of much greater consequence. But it has been shown by Becht and Greer, and by Hektoen and Carlson, that the titer of immune substances is much less in the fluids of the anterior chamber of the eye than in the blood, or it may be lacking altogether in the former when it is present in the latter region; and more recently, Appel, Saphir, Janota and Strauss have stated this to hold good also for immune substances produced by the growth of the Brown-Pearce tumor in rabbits. This condition would help

anterior chamber of the eye is indicated by the fact that heterogenous testes and ovaries survived only up to 20 days, and that at a later date only fibrous tissue was found.

The ovary seems to behave after intra-ocular transplantation in a similar way to the testis (Goodman, as well as Lane and Markee); however, the various constituents of this organ are, on the whole, more resistant than those of the testis. In the case of both of these organs, hormones may affect not only the transplants, but the latter also give off hormones which may leave the eye and affect distant organs. In intra-ocular transplants of seminal vesicles and prostate of the rabbit, R. A. Moore and his collaborators have found that the effects of repeated stimulation of the transplants by hormones follow a definite curve; the growth response is strongest in the beginning and then soon declines.

In intra-ocular transplantations of the adrenal gland, conditions are in principle similar to those of the testicle, as a comparison of the results of adrenal transplantations into the eye (Turner) and elsewhere (Wyman and Tum Suden, Atwell, Ingle and Higgins) indicate. Here also, stimulation by the specific anterior pituitary hormone which occurs especially in adrenalectomized animals, is important. However, under the more favorable conditions existing in the anterior chamber of the eye, cortical glomerulosa tissue may grow, differentiate, and survive for a long time, even in non-adrenalectomized animals; in these experiments, also, the organs of very young animals were used for grafting. But the stimulation by the pituitary hormone in adrenalectomized animals, or the repeated transplantation of pituitary lobes, enhanced the growth and the percentage of survivals.

Likewise, after intra-ocular transplantation of the hypophysis the grafts remain well preserved. The different types of hypophyseal cells are affected in the usual way by various hormones, and, conversely, transplants of the hypophysis through their own hormones may affect other organs (R. M. May, Haterius, Schweizer and Charipper, Martins); but as mentioned previously, pituitary transplants survive for a long time also after subcutaneous transplantation in mice, if the individuality differentials of host and donor are relatively harmonious.

So far, we have studied only the fate of intra-ocular transplants of tissues which were very young or were derived from litter mates and which had, therefore, special advantages. However, in order to differentiate between the factors which distinguish the reactions against strange individuality differentials in the anterior chamber of the eye and in the subcutaneous tissue, it is necessary to transplant into the eye adult tissue and, preferably, thyroid gland. We carried out heterogenous as well as homoiogenous transplantations of rat thyroid, into the eye of the guinea pig. Living homoiogenous thyroid tissue was found at various times from 20 to 50 days after transplantation. There was a diminution of both the intensity of the connective-tissue and the lymphocytic reaction against the transplant, which for this reason may have shown a slightly better preservation. But neither invasion by fibrous tissue nor lymphocytic infiltration was entirely lacking in and around these transplants. After

heterogenous transplantation of thyroid from rat to guinea pig, living acinar tissue was found in two cases 10 days, and in two other cases, 18 days after transplantation; there was formation of hyaline connective tissue in and around these heterogenous transplants, and in some instances polymorphonuclear leucocytes collected around them. At dates later than 18 days, the transplants had disappeared. In this case, also, the results were somewhat better than in heterogenous transplantations into the subcutaneous tissue.

If we compare with these results, those obtained after intra-ocular transplantations of malignant tumors, the latter are much more striking. In a number of instances it has been possible to obtain active, continued growth in the anterior chamber of the eye, where none was found subcutaneously, and even heterogenous tumors, including human tumors, grew, in contrast to the subcutaneous grafts of this tissue. The difference between the growth of tumors obtained in these two sites is much greater than that of normal adult tissue. Results of this kind have been recorded by Smirnova, by Greene, by Greene and Saxton, by Appel, Saphir, Janota and Strauss, and by Cheever and Morgan; but Greene found that not all heterogenous tumors could be successfully transplanted into the eye; and the degree of success seemed to depend upon the original growth energy of the tumor used for transplantation. In some instances, also serial transplantations in the eye were successful. After retransplantation to the subcutaneous tissue, of tumors that had grown in the eye, the tumor cells died. The greater power of survival and growth of tumors in the anterior chamber of the eye as compared to that of ordinary tissues is in part probably due to the greater growth momentum inherent in tumors, which leads to a multiplication of the advantages offered by conditions in this site as compared with those present in the subcutaneous tissue. However it is possible that still another factor is active, namely, the diminution or lack of immune substances in this region. Tumors, as a result of their growth in hosts bearing different individuality differentials, as a rule seem to give rise to immune processes to a higher degree than do normal tissues, perhaps at least partly on account of their increase in mass, which takes place with relatively great rapidity, and tumors are very sensitive to the injurious action of such substances, especially during the first period following transplantation. In the case of ordinary tissues, as we have seen, the primary homoio- and heterotoxins are apparently very much more important in the determination of their fate after transplantation than are the immune substances, although the latter may play some role also; however, in the case of tumors there is evidence that though the primary homoio- and heterotoxins likewise help to determine the result after transplantation, the immune substances are of much greater consequence. But it has been shown by Becht and Greer, and by Hektoen and Carlson, that the titer of immune substances is much less in the fluids of the anterior chamber of the eye than in the blood, or it may be lacking altogether in the former when it is present in the latter region; and more recently, Appel, Saphir, Janota and Strauss have stated this to hold good also for immune substances produced by the growth of the Brown-Pearce tumor in rabbits. This condition would help

anterior chamber of the eye is indicated by the fact that heterogenous testes and ovaries survived only up to 20 days, and that at a later date only fibrous tissue was found.

The ovary seems to behave after intra-ocular transplantation in a similar way to the testis (Goodman, as well as Lane and Markee); however, the various constituents of this organ are, on the whole, more resistant than those of the testis. In the case of both of these organs, hormones may affect not only the transplants, but the latter also give off hormones which may leave the eye and affect distant organs. In intra-ocular transplants of seminal vesicles and prostate of the rabbit, R. A. Moore and his collaborators have found that the effects of repeated stimulation of the transplants by hormones follow a definite curve; the growth response is strongest in the beginning and then soon declines.

In intra-ocular transplantations of the adrenal gland, conditions are in principle similar to those of the testicle, as a comparison of the results of adrenal transplantations into the eye (Turner) and elsewhere (Wyman and Tum Suden, Atwell, Ingle and Higgins) indicate. Here also, stimulation by the specific anterior pituitary hormone which occurs especially in adrenalectomized animals, is important. However, under the more favorable conditions existing in the anterior chamber of the eye, cortical glomerulosa tissue may grow, differentiate, and survive for a long time, even in non-adrenalectomized animals; in these experiments, also, the organs of very young animals were used for grafting. But the stimulation by the pituitary hormone in adrenalectomized animals, or the repeated transplantation of pituitary lobes, enhanced the growth and the percentage of survivals.

Likewise, after intra-ocular transplantation of the hypophysis the grafts remain well preserved. The different types of hypophyseal cells are affected in the usual way by various hormones, and, conversely, transplants of the hypophysis through their own hormones may affect other organs (R. M. May, Haterius, Schweizer and Charipper, Martins); but as mentioned previously, pituitary transplants survive for a long time also after subcutaneous transplantation in mice, if the individuality differentials of host and donor are relatively harmonious.

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to explain the special advantage which the eye offers for the growth of certain transplanted tumors, as compared with the subcutaneous tissue or other sites. Against such an interpretation are the recent experiments of Greene, as well as those of Cheever and Morgan, which indicate that a transmission of immune processes may take place between the aqueous humor of the eye and the circulating blood, although again, the results of these investigators, working with different types of tumors, differ as to the degree to which such an exchange may occur. Moreover, as Greene points out, the growth of tumors is enhanced if the testicle is used as the site of transplantation, and in this organ such a barrier between blood and organ constituents does not, in all probability, exist. Also, in other kinds of transplantations the testicle has been found to be a favorable site; thus, according to Stockard, homoiotransplantation of the ovary in the salamander Diemyctylus, succeeds only in the testicle; likewise, the Pearce-Brown rabbit tumor is advantageously propagated by grafting it into the testicle. The most probable conclusion in regard to immune substances, at the present time, seems to be that a certain degree of interference with the exchange of these substances between blood and the fluids of the eye may play some part in favoring the growth of tumors in the anterior chamber of the eye, but it may not be the only factor concerned. However, it is possible also that the primary homoio- and heterotoxic substances may pass only with greater difficulty from the circulating blood or lymph into the fluid of the anterior chamber, and that this condition may contribute to the advantage which transplantation of tumors into the eye has as compared to the subcutaneous tissue; furthermore, fluid in this site surrounds a part of the periphery of the transplant and this may favor a continuous removal of individuality differential substances functioning as homoio- or heterotoxins, from the transplant. But, as stated, there exists the further possibility that still other, as yet unknown, factors may cause a mitigation of the injurious reaction of the host against transplants in special locations, including the anterior chamber of the eye.

Transplantations into the eye have contributed additional information as to the effect of hormones on the survival and growth of transplanted endocrine organs. By means of these transplantations, more evidence has been obtained for the conclusion that various secreting cells living under unfavorable conditions, may not be able to sustain themselves without receiving stimulation by specific hormones; a certain degree of disharmony between the individuality differentials of host and transplant may be one of these unfavorable conditions. Strange individuality differentials bringing about degenerative effects in the transplant, as, for instance, in the ovary and adrenal cortex may help to induce connective tissue cells as well as lymphocytes to react very strongly against tissues bearing these strange individuality differentials, in the manner already indicated in the discussion of transplantations of ovary and adrenal gland in the mouse. By inhibiting or preventing these degenerative alterations, hormones may protect the transplanted tissues against these intensified reactions, especially of lymphocytes. It has likewise been shown that the activity of the connective tissue providing the stroma of organs is

partly conditioned by the activity of the epithelial tissues. The latter therefore help to determine whether the stroma shall be cellular or fibrous in character; by stimulating the function of the parenchymatous tissues, hormones may thus indirectly also affect the character of the stroma. It may then be stated again that one of the factors which aids in the survival and function of certain differentiated and therefore sensitive endocrine tissues, is not so much a deficiency in function of the corresponding host organ, as a stimulation of the transplant by the effective hormone of the host. The deficiency required may be merely a means of accomplishing a stimulation of the transplant by the hormone.

In conclusion, it follows from the data discussed in this chapter that in certain organs of the adult host, or in embryonic structures, various special conditions exist, which protect at least to some extent tissues possessing strange individuality or species differentials from the injurious action of the bodyfluids and cells of the host, but that the nature of these mechanisms is, at the present time, only imperfectly understood.

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which are characteristic of older age in various organs. Carrel has found that the blood serum of older animals is less suitable as a medium in which tissues grow in vitro than is that of younger individuals. However, it is not certain that this factor plays a significant role in the living organism.

We approached this problem by means of transplantation of the thyroid gland in guinea pigs. Our observations showed that within the first 10 days of extrauterine life of the host the connective-tissue and lymphocytic reactions against homoiogenous thyroid gland are less intense than in adult hosts. Tureen then compared, in our laboratory, transplantations of thyroid glands in which adult guinea pigs were the donors and in which the donor-age was therefore constant, while the age of the host varied. In the group of young hosts the age varied between 4 days and 5 weeks, while in the group of older hosts variations in weight between 500 and 800 grams indicated corresponding variations in age. In the first 4 or 5 days the reaction was about the same in both groups. But from then on a difference developed: in the older guinea pigs there was a more marked formation of fibrous tissue, which destroyed a considerable part of the transplant, and, in the majority of cases, destroyed it entirely after 20 days or more had elapsed. In the younger animals the formation of the fibrous tissue was considerably less in most of the animals and the thyroid tissue was therefore better preserved. But in a certain number of instances there was a marked fibrous reaction also in the younger guinea pigs. However, because in the majority of the younger group the preservation of the gland was so much better, the homoiogenous individuality differentials were here subsequently given off in larger quantities; these then attracted the lymphocytes, which, somewhat later, surrounded and invaded the transplant in considerable numbers and, secondarily injured it. In younger animals, as a rule, there is therefore a tendency for the homoiogenous tissue to elicit a syngenesio rather than a severe homoiogenous reaction. A combination of homoiogenous individuality differentials, and a relatively older age of the host, led thus to an early increase in the formation of fibrous tissue in or around the transplant. Under the influence of not well compatible individuality differentials the stroma developing in the transplants in adult animals is inclined to assume prematurely the fibrous character which is so characteristic of the bodily structures in old age. Because of these injurious effects grafts in older hosts were, then, less liable to attract lymphocytes than the better preserved tissues of younger animals. But when in older guinea pigs the preservation of the thyroid tissue happened to be better, in such animals, also, a larger number of lymphocytes were attracted; hence it is the strong connective-tissue reaction in the older animals which in these instances caused the difference in the fate of the graft in the old and in the young guinea pigs. Whether an increased toxicity of the bodyfluids in older hosts contributed to the accelerated and intensified injury of the graft is difficult to determine, because the injury by the connective tissue was so marked that it might have obscured a damaging effect of the bodyfluids. We have already remarked that in old mice, transplants of thyroid and cartilage and fat tissue could do as well as in younger animals.

Chapter 19

The Relations Between Age and Individuality Differentials

THE FRENCH surgeon, Ollier, observed, during the latter half of the last century, that autotransplants of skin and periosteum grew much better in young than in older individuals, where they grew only temporarily. Also, Schoene noticed that old age is unfavorable for transplantation of skin and that in old rats even autotransplantation may yield bad results. Kozelka found, in transplantation of skin into fowl, that the adult host had greater resistance to grafts of strange skin than the chick and that also the adult tissue is less able to adapt itself to an adult host than the young tissue to a very young host, and furthermore, that young grafts in young hosts remain alive or regress only slightly when the host becomes older. He assumed that the milder form of tissue antagonism present in the host enabled it to eliminate the incompatible elements, without totally destroying the tissue. According to Pfeiffer, the gonads of immature animals, and especially those of immature rats, take more readily than those of adult rats. On various occasions we have compared the reaction against strange grafts in young and in older rats and guinea pigs. In the young, inbred King rats the reaction against transplants of various tissues was milder than in older rats, and not only against transplants within the inbred strain, but also against those from hybrids, in which latter a constituent had entered which was strange to the member of the inbred parent strain serving as host. In experiments in mice we had observed that in somewhat older mice the reaction against the transplant was, in certain cases, stronger than in very young mice, although this did not need to be the case in all experiments. In older mice, from 10 months to 20 months old, transplants of various tissues from younger animals could be as well preserved as in younger hosts, and the reaction was not noticeably more severe in these old mice than in younger adult mice.

There remains the problem as to the mechanism by means of which age affects the transplants and in this respect experimental evidence is as yet slight; it will be necessary especially to consider separately the effect of age on the host and on the transplanted tissue. If even in autotransplantations, skin and bone grafting is less favorable in older than in younger individuals, this is possibly due to the better vascularization and to the greater tendency of the connective tissue to remain more cellular and less fibrillar in younger organisms. This condition seems to be independent of the reaction of the individuals serving as donors and as hosts against strange individuality differentials; it is related, in all probability, to the fibrous changes in the stroma,

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As to the reason why transplantations from very young donors may succeed better than those from older ones, our knowledge is still less definite. However, by means of the white blood cell reaction Blumenthal could show that both tissues from early as well as from later stages of rat embryos elicited a lymphocytic reaction after transplantation into adult rats; but rat or mouse embryos obtained during the first half of pregnancy called forth, in a heterogenous host, merely an increase in lymphocytes, as an indication that the organismal differentials were not yet fully formed at this period; a short time before the end of pregnancy the typical heterogenous reaction did develop. In this connection, the fact must be recalled that also implantation of non-living protein substances may call forth a lymphocytic reaction and it is therefore possible that a non-specific or at least a less specific, factor caused the effect which very young embryonic tissue exerted on the lymphocytes of the host. It may then be concluded from these experiments that the organismal differentials are fully developed in newborn animals, and if tissues from very young donors survive better in homoiogenous hosts than those from older ones, this must be due to other factors than lack of differentials. In this regard we have to consider, in the first place, the greater growth momentum of the younger tissues, and perhaps also their greater adaptability to inadequate environmental conditions. The increased growth momentum may be able to overcome injurious conditions, which more slowly growing, adult tissues cannot overcome so readily. This view would be in harmony with the experience that transplanted rapidly growing tumors which possess a strong growth momentum may be more resistant to the action of unfavorable individuality differentials than normal tissues, and, similarly, embryonic tissue may be more independent of the action of such differentials.

There is still a further point to be considered. Certain organs from old animals show changes which make them less suited for transplantation than the corresponding organs from younger ones; thus the ovaries of older mice and of other species contain few follicles, and the thyroid gland in certain strains of mice undergoes sclerosis; these are conditions not favorable to a good development and function of the essential constituents of the organs when transplanted.

We have attempted in this analysis to separate the various factors which may cause the difference in the results in carrying out transplantation experiments, using young and old animals as hosts and donors, and we have found that the individuality differentials are fully developed in young donors and that a lack of the differentials is not one of the factors that causes the difference in the results of homoiotransplantation in animals of different ages. The greater tendency to the formation of fibrous tissue in older, homoiogenous hosts and the greater growth energy of younger tissues may explain at least some of these differences.

Chapter 20

Individuality Differentials and Tissue Culture

In the living organism, tissues bearing strange individuality differentials are injured by the homoio- or heterotoxins circulating in the bodyfluids of the host, as well as by the cells of the host. The relative importance of these two injurious factors differs in different species and with different tissues. In higher organisms, the reactions against tissues bearing homoiogenous individuality differentials are, as a rule, severe; however, if tissues are grown in tissue culture, no special difference in the effect of autogenous and homoiogenous serum or plasma serving as media is noticeable. This follows from the observations of I. T. Genther and the writer, which showed that the number of mitoses in the guinea pig thyroid was about the same in vitro in autogenous and homoiogenous serum, when it might be expected that quantitative differences in mitotic activity would serve as a delicate indicator of the injurious influence of homoiotoxins. Likewise, the differences between the effects of homoiogenous and heterogenous plasma or serum on tissue growing in vitro are much less than are the corresponding differences between the effects of homoiogenous and heterogenous hosts on tissues transplanted into living animals. Thus Lambert and Hanes noted that rat sarcoma cells may grow in guinea pig plasma for 30 days, in rabbit plasma for about 12 days, in dog plasma for 2-3 days, and in pigeon plasma for 4-5 days, but no growth of rat or mouse tumor cells took place in goat plasma. Also, motile cells of the spleen could grow out in heterogenous plasma, and both rat sarcoma and rat spleen produced giant cells in such a medium; a culture of fibroblasts remained active, for a certain time at least, in a heterogenous medium, but the injurious effect of heterotoxins became manifest more readily in normal fibroblasts than in certain tumor cells (A. Fischer). There may be active in these cases, both the strange organismal differentials, whose effect is graded in accordance with phylogenetic relationship, and special toxic substances, whose action does not correspond directly to this relationship.

The same two factors play a role also in amphibian tissues growing in vitro. Thus, Rhoda Erdmann cultivated skin of Bufo first in Bufo plasma and Bufo spleen extract, next in Bufo plasma and frog spleen extract, and in the end in frog plasma and frog spleen extract, by these means a gradual adaptation of tissues to strange organismal differentials was achieved. The skin of another amphibian species could likewise be cultivated in combinations of heterogenous plasma and tissue extracts. Hitchcock found that frog skin of a certain species grew equally well in autogenous and in homoiogenous plasma or serum, and also in the bodyfluids of heterogenous species of Rana. However, frog skin was rapidly killed in vitro by Necturus plasma

and Necturus skin was similarly affected by frog plasma. But skin of Necturus, as well as of Triturus, grew well in plasma and serum of Necturus. It may then be concluded that the differences in the reactions against other than autogenous tissues are much less when the tissues are grown in homoiogenous or heterogenous serum or plasma, than when they are placed in homoiogenous or heterogenous living hosts. However, it must not be concluded from these and other similar experiments that no differences exist, as far as tissues growing in vitro are concerned, between the effect of homoiogenous and heterogenous media; results obtained by Hitchcock already suggest that such differences do exist. Likewise, experiments with mammalian tissues indicate that homoiogenous plasmas and sera are preferable to heterogenous ones, although the admixture of heterogenous tissue extracts to such media seems not to interfere seriously with the life and growth of tissues under these conditions. Thus it has been possible for fibroblasts from the subcutaneous tissue of the adult mouse to grow actively for many successive generations in a culture medium of chickenplasma, chick embryo extract and horseserum without serious interference with the proliferation, motility and structural potentialities of these cells.

There exist various differences between tissues living in their normal environment, tissues transplanted into other living organisms, and tissues cultivated in vitro. In tissue culture, the aggressive action of host cells which attack the grafts is eliminated; in vitro the tissues are exposed merely to the action of homoiogenous or heterogenous organismal differentials, contained in the bodyfluids, and the toxic effect produced on them by the latter is less than when they are transplanted into living hosts.

As to the conditions which render these bodyfluids less injurious in tissue culture: (1) One factor is probably the small amount of blood plasma or serum present in the culture media, which contains the toxins, as compared to the continuous current of fluid carrying fresh supplies of homoio- and heterotoxins to the transplant in the living body. Such a condition may be active also when the homoiogenous plasma of an animal which had proved to be immune to the growth of a certain tumor, is used as a culture medium for a piece from the same tumor growing in vitro; it does not prevent the growth of the tumor; under these circumstances, the amount of homoiotoxins present at a certain time is presumably insufficient. (2) A second factor concerns the growth momentum of cells in vitro. Cells growing in vitro are not components of an ordinary, relatively resting tissue; they are very actively growing and are either of embryonal origin or are derived from adult cells, or, they may be cancer cells. Both embryonal cells and cancer cells are under the influence of factors which stimulate them to grow continuously, while cells derived from adult cells, being separated from their normal environment, are continuously regenerating. In all these types of cells the growth momentum is increased, and furthermore, it is possible that in the case of the embryonal cells the individuality differentials may not yet be completely developed. Such an increase in growth momentum makes it possible for these cells to overcome difficulties to which other cells might succumb; in

addition, they may lack certain products of differentiation which might serve as an effective point of attack for injurious substances present in the circulating blood; this is suggested by the fact that in actively growing cells, whether they are embryonal, regenerating adult, or tumor cells, there is less tendency to differentiation and a full development of the tissue or organ differentials is lacking—a condition noted apparently also in plant cells growing in vitro, as the experiments of White indicate. On the other hand, if factors capable of inflicting a limited degree of injury, act on these stimulated, actively-growing cells, either normal or abnormal processes of differentiation may occur, which, as an endstage, may lead to cell death. It seems that with this diminution in the development of tissue differentials and in tissue differentiation, as well as with the increase in growth momentum, there is perhaps associated also a diminution in the sensitiveness to not quite adequate individuality differentials. These factors, taken together, might then explain why tissues growing in tissue cultures are less affected by not quite harmonious individuality differentials than normal adult, relatively resting, differentiated tissues.

Chapter 21

The Individuality Differentials and Potential Immortality of Tissues

In the preceding chapter we have analyzed the significance of individuality differentials in the life of tissues growing in vitro and have tried to explain the relative independence of the cells living under these conditions from the nature of the individuality differentials and the diminution in the significance of the species differentials of the surrounding media. The same factors which are active under these conditions enable the cells to live and propagate indefinitely, provided definite experimental requirements are fulfilled. It could be shown that some cells and tissues of mammalian organisms are potentially immortal. This holds good, with the reservation that the term "immortality" is applied here in a relative, not in an absolute sense, the immortality being limited by the need of the existence of certain environmental factors, which in all probability will come to an end in some distant future.

However, the potential immortality of various mammalian tissues was first recognized in the case of tumors. In 1901, we showed that it is possible to transplant tumors through many consecutive generations of animals of the species or strain in which the tumor originated. There seemed to be no limit to the continuous life inherent in the propagated cells, inasmuch as the termination of these long continued serial transplantations depended solely upon accidental, unfavorable factors which could be avoided. Furthermore, since it was evident that tumor cells are merely ordinary tissue cells which could be transformed into tumor cells at will under well-defined experimental conditions, the conclusion was justified that also the normal cells from which the tumor cells were derived, have the potentiality to immortal life.

Subsequently, a second method, already mentioned, was used by Carrel and Ebeling, who transferred embryonic connective tissue cells serially from generation to generation in tissue culture. Here the embryonic cells are stimulated to multiply indefinitely by the conditions which have been prepared for them experimentally; when transferred serially to fresh culture media, they may be kept alive indefinitely. But while it is mainly embryonic fibroblasts which have been propagated in this way from generation to generation, there are a considerable number of types of normal cells which, after transformation into tumor cells, have acquired the ability to propagate indefinitely. This is true not only of different types of connective tissue cells, but of mammary gland tissue and various other epithelial cells; also of endothelial and cartilage cells; indeed, it is in principle true probably of all cells which constitute transplanted malignant as well as some benign tumors.

While, therefore, on theoretical grounds it is justifiable to extend the conclusion as to the potential immortality of cells to ordinary tissue cells, actually it has not been possible to demonstrate this characteristic by the same method in normal tissues as in tumors, on account of the more severe injurious effects produced by strange individuality differentials on normal tissues, as compared to tumors, after their transplantation into new hosts. Our attempts to transplant epidermis serially succeeded for only a relatively short period. Normal cartilage seemed to be a much more favorable tissue for long-continued transplantation, inasmuch as it is more resistant to injurious conditions than are most tissues, and better able to withstand the unfavorable effects of the homoiotoxins of the bodyfluids of the host and of the aggressive host cells, especially the connective tissue cells and lymphocytes. In addition, there is some reason for believing that transplanted cartilage gives off a smaller amount of homoiogenous substance than do other more actively metabolizing tissues. It was thus possible to transplant cartilage serially for several years, and not only into young rats but also into very old animals which were approaching the end of their life. In these experiments it was the xiphoid cartilage of rats which was transplanted into dorsal subcutaneous pockets of other rats. The length of time elapsing between consecutive transplantations of a piece of cartilage to a series of hosts varied between one month and one year. On the average, a new transplantation was carried out every five to six months. It was thus possible to keep the transplant alive for several years, since at the time of the first transplantation the cartilage had already reached an age varying between two and three years and it could be transplanted serially for more than three years; at the end of the experiment the cartilage had reached an age of five to six years, a period considerably exceeding the average length of life of the rat, which is usually not more than three years.

This relative success in the serial transplantation of cartilage is due to the factors mentioned above. The lymphocytes of the host accumulate around the transplant in smaller numbers, and, as stated previously, the lymphocytic reaction may even decrease in the course of time. The lymphocytes were found in the largest number in the fourth week, and from then on their number gradually decreased, until after five months the transplant showed usually only a very weak or no lymphocytic reaction; but 20 days after re-transplantation the lymphocytic reaction could again become distinct. In the course of these transplantations the perichondrium may regenerate and form new cartilage around a piece of this tissue, that had become necrotic as a result of transplantation. Groups of very young perichondrial cartilage cells may be found at the time of examination, but the new cartilage does not penetrate into the surrounding tissue. The perichondrium produces cartilage towards the inside, ensheathing or replacing the old cartilage, but towards the outside it seems to produce a tissue that is transitional between cartilage and fibrous tissue, and that resembles at the outermost border typical fibrous tissue. However, if the transplanted cartilage becomes thick as the result of the growth activity of the perichondrium—

and this is true even if normal, not transplanted cartilage has reached a certain thickness—the central parts may shrink and become necrotic, due to their distance from the blood vessels which carry nourishment to the perichondrium and to the outer layers of the cartilage. It is probably also the deficiency in oxygen and other food material that causes the small, relatively undifferentiated perichondrial cells to change into the large and fully differentiated cartilage cells. Only in one case had a piece of cartilage, after a period of serial transplantations extending over two years and ten months, produced bone; bone plates were lying along the cartilage and in the bone there was a development of marrow containing fat cells.

It is the connective tissue which is active in the destruction of serially transplanted cartilage. This forms a capsule around the transplant and it invades, dissolves and gradually replaces the necrotic parts; occasionally, blood capillaries and some lymphocytes may penetrate with the connective tissue into the areas of necrosis. But under certain conditions the connective tissue may push its way also into that part of the living cartilage where the cells are separated by a relatively large amount of hyaline intercellular substance or by a very thick capsule. On the other hand, the connective tissue is apparently unable to penetrate into living perichondrium or into young perichondrial cartilage, where the cells are placed close to one another. It is therefore the fargoing differentiation, the marked formation of intercellular substanee or of capsule meterial, which gives the conneetive tissue an opportunity to exert its invasive, constrictive, and therefore injurious action. Healthy young cartilage eells are safe from the attack by the host connective tissue, although, as we have seen previously, they are exposed to the invasion by lymphocytes. Thus a vicious circle is established: certain unfavorable conditions, such as deficient nourishment, lead to the production of the differentiated cellular and intercellular paraplastic substances, and then the resulting ingrowth of connective tissue tends to divide the transplant into small partitions and otherwise injure it, decreasing still further its normal oxygen and food supply and preventing its normal proliferation. To ensure the survival of the cartilage transplant, it is necessary to keep the perichondrium surrounding it alive. The tissue equilibrium is best maintained if the resting connective tissue of the host surrounds the perichondrium of the transplanted resting cartilage. But at the same time it is necessary to prevent the impairment of the nourishment of the transplant by the connective tissue capsule. If there is a deficiency in the nourishment of the transplant, a necrosis in its center occurs, the tissue equilibrium is disturbed, and in consequence the new formation of the perichondrial cartilage cells takes place, which subsequently differentiate and produce intercellular substance. Thus both (1) primary injurious conditions which affect directly the transplant, and (2) the activity of the host connective tissue and lymphocytes, taking place under the influence of homoiogenous individuality differentials, may play a part in shortening the life of the transplant. In old age, changes similar to those seen after homoiogenous transplantation occur in organs, namely, a decrease in the parenchyma and an increase in the

fibrous stroma. The primary degenerative changes in the parenchyma may stimulate the connective tissue or glia to increased activity. These changes in the stroma impair still more the preservation and functioning of the parenchyma, *which then may undergo further degeneration.*

However, if we select very closely inbred strains of mice, where all the individuality differentials approach the character of autogenous differentials—although this state has not yet been completely attained—the prospects of a successful serial transplantation even of whole organs such as thyroid gland are greatly improved. Moreover, in the mouse the host cells, whose function it is to attack the tissues possessing strange differentials, are often less active than they are in rat and guinea pig. Hence it has been possible in our experiments to prolong the life of serially transplanted organs beyond the usual length of life of the mouse, and there are indications that it may be possible, by carrying out serial transplantations in closely inbred strains, to keep alive and growing indefinitely not only cells which are more or less independent of each other, such as connective tissue cells, but also whole organs.

The potential immortality of mammalian cells has then so far been demonstrated by two methods, in both of which the cells are subjected to unceasing, intensified growth stimulation—namely (1) the serial transplantation of tumors and (2) the continued transfer of cells in tissue culture. In the first method specific tumor stimuli, and in the latter stimuli characteristic of regenerative and embryonal growth are active. The constant renewal of the cells by mitosis, under the influence of these stimuli, prevents undue differentiation and production of paraplastic substances, which would injure the cells and in the end prematurely destroy them. Cells which have gained in differentiation beyond a certain limit and, correspondingly, lost in the power of propagation, such as ganglia cells or certain leucocytes, either slowly undergo gradual atrophy or degenerative processes or they die at an early period. Cells which, as a result of processes of differentiation, have lost, not yet entirely but to a certain degree, their power of propagation, undergo abnormal changes of further differentiation when acted upon by growth stimuli originating from the outside or within the cells themselves. The same process may therefore function both as growth stimulator and, in a certain sense, also as differentiator of cells, if it acts on a cellular substratum in which an intermediate degree of differentiation has taken place. But, as stated, if the differentiation has reached a further advanced stage, growth stimuli may induce alterations in the cell equilibrium so great that they lead to cell death, which thus represents the endstage of the differentiating process.

While both methods, which have been used so far for the demonstration of the potential immortality of tissues of higher organisms, require the constant action of growth-promoting factors, there remains the possibility that certain organs, such as the thyroid gland, in which the units composing the organ are closed cell complexes, forming acini or similar structures, may through well-timed serial transplantation be kept alive *indefinitely,* without a very active cell proliferation taking place. This can, however, be accomplished only if the individuality differentials of the host and transplant

are very similar. Homoiogenous and heterogeneous individuality differentials represent one of the most important injurious factors opposing the perpetual life of tissues if separated from their normal connections. However, even within the animal's own body, where the individuality differentials are autogenous, the return to the original tissue equilibrium after a disequilibration has been established may be incomplete, owing to the fact that the various tissues live under environmental conditions to which they are not fully adapted and which, under some circumstances may become injurious. These factors, step by step, cause the old age changes and, finally the death of the tissues and of the individual in which, for a time, they have functioned. The cells have to live under such injurious conditions because they exert functions which concern the organism as a whole, and they are acted upon by cells and substances which likewise function in the interest of the whole organism; it is this condition which causes their ultimate destruction. Hence potential immortality does not apply to the higher organism as a whole, but only to certain types of cells or organs which constitute parts of this organism. The individual as such, as far as is known at present, cannot avoid death.

Chapter 22

The Nature of the Individuality Differentials and of the Reaction of an Organism Against a Strange Individuality Differential

We have discussed the reactions of the host against the various types of individuality differentials in various species of animals, but in every case we have dealt with reactions against tissues containing an individuality differential mixed with other substances, and not with the reaction of cells and tissues against an individuality differential isolated in a pure state. No direct attempt has been made so far to determine the chemical structure of this substance. However, by subjecting the tissues to various procedures, it has been possible to draw certain general conclusions as to the chemical constitution of the individuality differentials. We have used for this purpose (1) the effect of graded exposure of tissues to higher temperatures, and (2) the effect of different chemical substances on the individuality differentials present in various tissues. Tissues thus treated were then tested by means of transplantation into different types of hosts in the same way as normal tissues.

(1) *The effect of heat on the organismal differentials in* (a) *homoiogenous and* (b) *heterogenous tissues.* In experiments by Siebert, to which we have referred already, it was shown that by in vitro exposure of thyroid and cartilage of the guinea pig to temperatures varying between 43 and 51°C for half an hour and then by transplantation of these pieces into homoiogenous animals, it is possible to diminish very much the lymphocytic reaction of the host against the transplants. These results indicate that the heating at very moderate temperatures reduced markedly the quantity of homoiogenous individuality differentials which diffused from the graft into the surrounding host tissue. It is not certain whether in this case a definite injury of the individuality differentials had taken place in the transplanted tissues, or whether merely the diffusion of these differential substances into the surrounding tissue had been made more difficult.

It is more probable that the first explanation is the correct one, because a short delay in diffusion should not have affected so much the strength of the lymphocytic reaction, but should only have delayed its appearance for a short time. The heating of the tissues at the same temperature affected the reactions of a heterogenous host much less than those of a homoiogenous host; in the former there was only a slight diminution in the number of polymorphonuclear leucocytes and lymphocytes. This effect must have been due to a not very marked injury inflicted on the heterogenous differentials

through the heating. Blumenthal subsequently tested the effect of heating of both homoiogenous and heterogenous tissues on the organismal differentials by means of white blood cell counts. In these experiments, also, the tissues were exposed to heat for 30 minutes in a test tube previous to transplantation. A temperature of from 45° to 50°C hardly affected the individuality differential of guinea pig or rat thyroid, nor was the individuality differential of pigeon skin, which normally shows a weak reaction, much affected thereby. The same negative result was obtained when rat skin was heated at 52°C, but the individuality differential of pigeon thyroid, heated at 54°C, was weakened or destroyed in one-half of the experiments. This temperature seems to represent the critical point; but if the temperature reached 56°C, the individuality differential of thyroid in various species was destroyed, but that of the guinea pig kidney was merely weakened; presumably the denser texture of the kidney affords a better protection against the effect of the heat.

To test the heterogenous organismal differentials (species differentials), tissues were exchanged between rat and guinea pig. Heating at 56°C destroyed the heterogenous differentials of thyroid tissue of rat and guinea pig, but left those of cartilage and kidney unaffected. In kidney tissue, heating at 60°C destroyed the differentials in seven out of eight experiments, and in cartilage this temperature injured or destroyed the differentials in one-half of the cases, but heating at 65°C destroyed also the species differential in cartilage. In the experiments of Siebert, as well as in those of Blumenthal, the heterogenous differentials were somewhat more resistant to heat than the homoiogenous differentials. However, the temperatures needed for the injury of both types of differentials were somewhat lower in the experiments of Siebert, who used the local reactions as a test, than in those of Blumenthal, who made use of the blood-cell reaction. Furthermore, in the latter series the tissues possessing a denser texture were more resistant to the destructive effects of heat than those possessing a looser structure. While the differentials in thyroid were more sensitive than those in kidney, the latter were more sensitive than those in cartilage. This again corresponds to the gradation of sensitiveness of the various tissues to the action of strange differentials. The injury of the tissues takes a course parallel to that of the organismal differentials, which they contain. In general, we may conclude that the homoiogenous and heterogenous differentials possess marked sensitiveness to the injurious effects of heat, corresponding approximately to the heat sensitiveness of the organs of which they form a part and this sensitiveness may be attributed to the labile proteins which are the most characteristic constituent of living tissues. It seems probable therefore that the differentials themselves are proteins, or combinations of proteins with certain chemical groups of a different kind.

This conclusion is supported by experiments in which Blumenthal exposed tissues to the action of various chemicals and then tested their effect on homoiogenous differentials by the alterations induced by these differentials in the white blood counts of the host. Least injurious for the individuality differ-

entials was glycerine or a 0.9% solution of sodium chloride, saturated with thymol. In the latter, the individuality differentials were active in the majority of cases after immersion for 24 hours, and in the former, after immersion for 12 hours. In acetone and in half molar sodium benzoate, the transplants in most cases were inactive after immersion for one hour, but some remained active; after 12 or 24 hours immersion, they all had become inactive. In 95% alcohol, 37% formaldehyde, and one-half saturated solution of ammonium sulfate, all the pieces were inactive after immersion for one hour. Treated with 50% urea, less than one-half of the pieces were active after immersion for one hour; with one-half molar ferrous chloride and with 1/200 molar ferrous chloride for six hours, all the pieces had become inactive.

The substances used in these experiments may act in either of two ways: (1) some may extract the homoiogenous differentials, and (2) others, especially those which affect the proteins, probably injure or destroy the homoiogenous differentials. Among the latter, most effective are those substances which actively denature protein. In general, the homoiogenous differentials proved to be very sensitive to chemical substances of various kinds and particularly to substances which alter proteins. There is reason for assuming that these differentials are produced only in living tissues, inasmuch as the majority of the pieces of tissues which had become ineffective, after having been subjected to the action of such a chemical, had been killed or severely injured. If, under these conditions, merely a newformation of the individuality differentials had been prevented, it should have been possible at least for the differentials preformed in the tissues to be potent. However, as stated, it seems that the majority of the chemicals used injured also these individuality differential substances as such.

It is of interest to compare with these reactions, those in which Blumenthal introduced various proteins, carbohydrates, fats, or lipoids subcutaneously. Substances which were liquid were injected subcutaneously on successive days. The proteins caused a reaction in the peripheral blood, similar to that induced by homoiogenous differentials. But it differed from the latter reaction in that the response of blood cells was not destroyed by a preliminary exposure of these protein substances to heat. Heterogenous rabbit serum and heterogenous embryonic tissue behaved like these protein substances; they elicited merely an increase of lymphocytes in the blood, which appeared between the second and fourth day after implantation of the foreign protein. None of the non-protein substances gave rise to this reaction.

From these experiments, it follows that as far as the effects of strange substances on the white blood cells circulating in the blood vessels indicate there is a definite order in which these substances can be arranged. (1) The finest gradations in the reactions are found if substances possessing strange individuality or species differentials are introduced. The reactions here correspond to the genetic relationship between host and donor. (2) Next come substances of a protein nature, which cause reactions not unlike those produced by homoiogenous differentials; the former elicit more marked reactions than those brought about by autogenous individuality differentials, which themselves

induce no changes, the changes noted being due merely to the operation on the animal. But these protein substances continue to exert their effects after they have been subjected to treatments which deprive homoiogenous differentials of their characteristic influence. (3) Likewise, protein substances, such as those present in blood serum of strange species, exert a homoiogenous effect; (4) on the other hand, blood plasma of a different species exhibits the effects of true heterogenous differentials, probably because of the presence of fibrinogen. (5) Lastly, there are strange substances of a non-protein nature; these are devoid of any specific action and behave like autogenous substances. But again, in contrast to autogenous substances, non-protein substances are non-specific.

It might be of interest also to compare the local reaction elicited by ordinary foreign bodies with that induced by substances carrying autogenous, homoiogenous or heterogenous organismal differentials. The material which possesses individuality or species differentials, after introduction into the subcutaneous tissue, exerts a combination of two effects: (1) non-specific foreign body effects and (2) specific effects elicited by the individuality and species differentials

It will therefore be necessary to analyze the differences between the reactions against a foreign body and the more specific reactions against living tissue. The reactions of the host cells against the foreign body do not show those fine gradations which are elicited by the individuality and species differentials. Variations observed in the reactions against foreign bodies depend largely upon mechanical factors which distinguish different kinds of material, but there are also some slight differences in the mode of reaction shown by different host species. Common to all these foreign body reactions is the prominent part played by the connective tissue cells of the host; they move towards the strange material and attempt to invade and to make it into a part of the host by transforming it into fibrous tissue. They first surround the periphery and then turning at a right angle invade it, branching off in tree-like fashion; they act as though they were stimulated and activated by the foreign material. In contrast to the farther distant connective tissue, which tends to assume a resting condition, forming collagen fibers, the connective tissue directly adjoining the foreign body remains cellular. However, the soil into which these host cells have penetrated is injurious to them and after a certain time they are apt to perish; their fibroplasm and nuclei become admixed to the foreign material, such as 1% agar, and thus a new substratum consisting of a combination of disintegrating cellular material and the fibroplasm-agar-mixture is produced; into this other host cells penetrate and ultimately accomplish its organization. In the peripherpy of the foreign body and also in its fissures there may also collect giant cells of various sizes, and mononuclear cells, the nature of which is uncertain, but which may perhaps represent either modified connective tissue cells or monocytes. It seems that giant cells with their accumulation of nuclei and their increase in cytoplasm form especially in those places in which there is an obstacle to the progress of the host cells, as in furrows in the foreign body. All those cells, the giant cells as well as the mononuclear cells, or cells transitional between these two, possessing two

nuclei, may act as ameboid cells and as phagocytes; they send pseudopods into the foreign material, take small particles of it into their cell-body and if possible digest them, as indicated by the intracellular vacuoles to which they give rise. The cytoplasm of the giant cells may become quite vacuolated and in the end, the latter perish. Small particles of the foreign body, detached from these aggressive cells, may be found also between the connective tissue fibers surrounding the agar or filling the fissure in the agar which they had produced. In certain cases, but not very frequently, also lymphocytes accumulate round the piece or in its fissures; they were found more often in rat and guinea pig than in pigeon, although lymphocytes occur in the circulation in larger proportion in birds than in rodents and although they were more numerous around and in homoiogenous tissues in the pigeon than in guinea pig or rat. Their presence around foreign bodies is due to non-specific irritations which the foreign material exerts and perhaps to some as yet unknown accidental factors. Occasionally also polymorphonuclear leucocytes penetrate, likewise in tree-like fashion, into the agar and dissolve it readily; here they perish after some time and the dissolved material is ultimately replaced by ingrowing connective tissue. It is not certain whether invasion by these leucocytes is the result of an accidental infection by bacteria or whether it is due to other factors. If coagulated egg-white instead of agar is implanted into the subcutaneous tissue, the reactions of the host cells are, in principle, the same as with agar; but on account of the greater hardness of this substance, it is much more difficult for the cells to penetrate it and the reactions, therefore, take place for the most part in the periphery of the egg-white, but, to some extent, cells do invade it. Formation of fibrillar connective tissue capsules around the pieces seems to be relatively the more prominent the harder the material.

We see, then, that the same elements participate in these reactions against foreign bodies as against living tissues possessing individuality differentials and species differentials. But as stated, the local reactions against foreign bodies do not show the fine gradations in activity of the host cells which the homoiogenous and heterogenous tissues call forth. There is, here, on the whole, a marked rigidity noticeable, although the degree of participation of lymphocytes and polymorphonuclear leucocytes is very irregular and apparently due to factors which cannot be foreseen or are unknown, in contrast to the definite orderly manner in which these cells react against the autogenous, homoiogenous and heterogenous differentials of living tissues.

In contrast to these non-living substances and to tissues that have been killed by heat or by chemical substances, living tissues possess individuality and species differentials which call forth definite graded reactions on the part of the host cells and bodyfluids. However, as we have seen, indirectly also the tissue differentials of the grafts may under certain conditions partake in these specific reactions, either intensifying or weakening them. The character of different tissues belonging to the same donor may affect the reaction against strange individuality differentials: (1) by variations in the amounts of individuality differentials which they give off; (2) by the differences in the

strength of resistance which different transplanted tissues oppose to the injurious actions of the bodyfluids and cells of a host possessing different individuality differentials: (3) by the production of certain degenerative changes in the tissue, which may increase very much the strength of the lymphocytic reaction of the host; such an effect we have observed especially with transplants of ovarian and adrenal-cortical tissue; in addition, certain specific reactions may take place between two adjoining autogenous tissues within the same organism, if the equilibrium between these tissues is disturbed. Such reactions may, for instance, take place between autogenous grafts of pigmented skin into defects of white skin in the guinea pig, and similar reactions may occur between the squamous epithelium of the cervix and the cylindrical epithelium of the uterus, particularly under the influence of estrogenic stimulation.

Other factors of a secondary nature exist, which may affect the strength and character of the reactions of the host against organismal differentials, such as age of host or donor and stimulation leading to an increase in the growth momentum of the transplanted tissue. This increase enables the graft to overcome various obstacles present in the host and especially also an unfavorable constitution of the individuality differentials. In particular, hormones which stimulate the transplanted cells to grow may enable them better to withstand the attacks of the host. There may be associated with this increase in growth momentum a diminished differentiation of the transplanted tissue, which may also indirectly contribute to the decrease in the severity of the reaction of the host against the transplant. We have seen furthermore that there are locations in the host where the transplants are, to some extent, protected against the host reactions, and different mechanisms of these various kinds may be active in different places.

As to the character of these organismal differentials, they are essentially genetically determined, but they are not identical with the genes; they are gene derivatives which lead to the production of the chemical substances characteristic of these differentials. While the genes in the donor of the graft strange to the host are mainly responsible for the intensity of the host reaction, there are some indications that also genes in the host which are not present in the transplant, may play a certain role in this reaction. However, it seems that in addition the ability of the host to react against strange individuality differentials may vary. The mechanism underlying this difference between different hosts has not been determined, and it cannot be excluded at present that the function of certain organs or tissues, such as the reticulo-endothelial system, is involved. Besides, different species to which the hosts belong differ quite distinctly in the mode of their reactions against a strange individuality differential.

The character of the individuality and species differentials directly or indirectly determines the mutual compatibility or incompatibility of two different organisms; but there does not exist a simple reciprocal relation between the reactions of individual or species A to individual or species B or vice versa, but it follows from what has been stated, that A may differ from B in

its functions as host or donor. Furthermore, while the organismal differentials exert primarily a direct effect, in determining the reactions of the host against the transplant, secondarily they may function also as antigens and call forth immune reactions, which may contribute to the intensity of the reactions, although to an unlike degree in different tissues. As to the number of genes which are involved in these reactions, it is in all probability very large, as especially the experiments with closely inbred strains suggest. There is no indication that the genes determining the four primary blood-group differentials are the genes which determine the nature of the individuality differentials; however, if we consider also the large number of additional differentials already found in erythrocytes by means of agglutination or hemolysin reactions,—a number which will probably increase still more in the future—, it appears possible that the gene sets from which the blood cell antigens develop and the individuality differentials will more and more tend to approximate each other.

In this connection it may be stated that a distinction should be made between the terms "individual differential" and "individuality differential." The former may be regarded as the more general term, including many characteristics, which differentiate one individual from another, such as color of skin, hair, eyes, size, shape of body and its parts, psychical attributes and which comprise thus the organ and tissue differentials. In contrast to these, "individuality differential" is a more specific term, designating a definite characteristic which is common to all or most parts of one individual and which differentiates him from the common characteristic denominator in another individual.

There has evolved, as the result of a long-continued series of experiments, the concept of individuality and species differentials, and, in general, of organismal differentials, in their interactions with tissue differentials and various other factors characterizing the organism. This evolution was a gradual one, taking place step by step, in close connection with concepts which were prominent at certain periods in the development of biology and pathology. We may distinguish essentially four periods in this development: (1) In the first period transplantations among more primitive invertebrates had proved the importance of the polarity of tissues and of other structural peculiarities affecting the harmony between grafted parts and the host, and these observations were generalized and applied also to transplantations in higher vertebrates; (2) In a second period the discoveries concerning active immunity and anaphylaxis, and especially those concerning agglutinins, hemolysins and precipitins, very strongly influenced the interpretation of all subsequent experiments in transplantation; (3) Later Mendelian concepts of heredity, following the revival of the study of genetics at the beginning of this century, suggested that the results of grafting were determined by the presence or absence, in the host, of genes which the grafted tissue needed for survival; (4) The foregoing data, as far as they were found valid, and the addition of new experimental data gave then origin to the concept of the various types of organismal differentials as determiners of the mode and intensity of interaction between different organisms and their parts.

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Part II The Phylogenetic and Ontogenetic Development of Individuality and Organismal Differentials

Chapter 1

Transplantation and Individuality in Coelenterates and Planarians

IN THE FOREGOING part we have discussed the organismal differentials and their relations to organ and tissue differentials in the very complex, phylogenetically higher organisms; in a subsequent part we shall discuss these differentials also in certain pathological growths, which develop in vertebrates under abnormal conditions of stimulation. We now intend to undertake the same analysis in normal invertebrates and lower vertebrates. In each class of animals we wish to determine how far transplantation of parts of organisms indicates the presence of organismal differentials and what the relations of the organismal differentials are to the organ and tissue differentials.

In adult birds and mammals there is a very strong reaction against strange individuality differentials, and against strange organismal differentials in general; the normal equilibrium is strictly autogenous; it depends upon the presence of the same individuality differential in all the important tissues and organs, and it is disturbed and leads to notable reactions if small parts of tissues possessing a strange individuality differential are introduced into the animal body. The strong cellular reactions of the organism against interferences with its structural integrity indicate that this tissue equilibrium is relatively fixed and rigid. The replacement of lost parts by the organism is very much restricted and the reactions which take place, ultimately tend to maintain or restore the characteristic structural pattern of the individual. Associated with this fixity is the great complexity and differentiation in the tissues of each individual, which does not allow fargoing adaptations to new environmental conditions or a newformation of lost parts. The individual represents, therefore, a rigid autogenous equilibrium between its constituent parts.

It was of interest to determine whether this association between the degree of sensitiveness to and of reactivity against strange organismal differentials, [illegible]

a causal relation exists between these two sets of factors.

The special conditions confronting investigations in these more primitive organisms have made necessary in many cases different methods of experimentation. Instead of transplanting small pieces of tissues or organs, a method

The means which the animal organism possesses for the regulation of disturbances, initiating reactions tending to reestablish a state of *equilibrium* between the parts of an individual, are relatively limited. Against a great variety of interferences which may affect an organism, the latter reacts in a very similar manner making use of the small number of reaction patterns which are at its disposal, in accordance with its inherited constitution. The variations in the environmental agents which act on the organism are very much greater than the various modes of reaction which the affected individual can initiate, but a gradation in the kind and intensity in the individuality differential reactions which may take place against these interfering elements can be noted. No reaction occurs against living parts of the animal's own body, to which a complete adaptation exists. Likewise, against non-living constituents of the environment, other than protein substances, no general or specific local reaction occurs. Against dead protein substances the lymphocytes in the circulation, and presumably those of the lymphatic organs, react in about the same way as against homoiogenous individuality differentials, although locally there is a distinction between the reactions against living homoiogenous tissues and against dead material. The local reaction against all foreign bodies, whether of protein or non-protein nature, is in principle the same, tending to destroy, to transform and to incorporate the strange material into the body in a way which is least injurious to the organism. However, certain heterogenous material, non-living but containing formerly living elements such as blood clots, if introduced into the organism may call forth a local reaction corresponding to that seen after implantation of heterogenous living tissues. Also, the general blood-cell reaction is stronger against more complex heterogenous proteins than against the simpler ones. Thus injections of heterogenous blood sera elicit merely a lymphocytic reaction, whereas, subcutaneous implantation of more complex proteins, such as heterogenous fibrinogen induce a typical heterogenous blood-cell reaction.

There are thus increasing intensities and specificities of reactions noted if non-living substances of different degrees of chemical complexity are introduced into the body; but the maximal specificity in reaction is attained only if living tissues, bearing strange organismal differentials, are transplanted. It is therefore those substances which are most nearly related to the characteristic constituents of living tissues namely the most complex proteins which call forth reactions most similar to those elicited by the tissues themselves.

We see, then, that the reactions against strange individuality and species differentials are not entirely disconnected and newly created responses of the organism against interferences, but they represent the endstage of a series of interactions which are graded in specificity in accordance with the increasing complexity in the structure of the strange environmental elements, and in accordance with the increasing similarity between their constitution and the constitution of living tissues.

The existence of a rudimentary differentiation in coelenterates is well exemplified in the experiment of Burt, who showed that rings taken from the anterior pole have, after transplantation, a greater tendency to form heads than have foot pieces, which latter have a greater tendency to form a foot, although in exceptional cases here, also, a rudimentary head formation with tentacles can be induced. This predifferentiation can be overcome under certain conditions: (1) By the application of various external factors. In this way Jacques Loeb first produced heteromorphosis in Tubularia. (2) Also by the action of factors present within the organism; namely, when certain differentiated areas, in some cases strange organs, introduced by means of transplantation, extend their influence to other areas. Here we must again distinguish two sets of factors: (a) The action of contact substances, or—to use a more general term—contact mechanisms. Through contact with a differentiated transplant, as, for instance, with the head of a Hydra, the anterior pole or the foot region of the host can be induced to form, at or near the place of contact, an organ corresponding to the transplanted head. In this case we must assume that contact mechanisms (contact substances) induce a heteromorphosis, inasmuch as a new formation takes place at a point where normally another part of the organism would have developed. Thus, a head may be induced to form in a place where normally a foot had been, or it may form in the middle zone. The resistance to such a head formation is greater at the aboral part than at the oral part, owing to the predifferentiation of the host organism. We have, here, to deal with an organizer action comparable to that which plays so important a role during embryonal development. It is especially transplantation of a regenerating hydranth, but also of other kinds of tissues, such as parts of Hydra buds and peristome, which in a specific manner induces the formation of hydranths in Hydra viridis (E. N. Browne, Goldsmith). In addition to these factors which thus lead to the formation of supplementary organs, there may be active another factor, which consists in the tendency of an organ to inhibit the formation of an organ of the same kind, especially in its close proximity. From such a near point this inhibiting influence may be transmitted to more distant parts, but apparently with decreasing intensity. (b) Factors of a regenerative or restitutive character. These may tend to supplement a part of an organism which has been separated from the rest, in such a way that the formation of a whole organism results. In this latter interaction a more differentiated tissue again is generally more potent in determining what organs shall be produced, than one less differentiated—it is the dominating, directing constituent of the organism. Moreover, a larger part usually prevails over a smaller part, other conditions being equal. While the organizer action in coelenterates mentioned above may lead to the reproduction of the same organs as are present in the organism, the regenerative tendency on the other hand leads to the newformation of supplementary, therefore of different, organs or areas. There is another, more definite difference between the mode of action of such an organizer and of the integrative restitutive or regenerative factors. The former acts, as stated, presumably through contact substances or contact mechanisms, while the

commonly used in the case of higher organisms, in the more primitive animals, as a rule, larger parts are joined together. In some instances they are so large that the procedure is comparable to parabiosis rather than to ordinary tissue transplantation, except that in the typical parabiosis the size of the area of union between the two partners is usually much smaller than in transplantation as practiced in lower organisms. However, it is not only the size of the pieces joined together which suggests a comparison with parabiosis, but also the fact that in invertebrates parts of organisms have, on the whole, a much greater capacity to carry on an independent life and to restitute the whole organism than the corresponding pieces in vertebrates. The pieces to be joined together are therefore usually more independent of each other and more self-sufficient than is the case in ordinary transplantation in higher organisms. We might also express these differences by distinguishing between organismal transplantations in which organisms or parts of organisms capable of independent life and of restitution into whole organisms are joined together, and tissue or organ transplantations in which the transplants are devoid of such capabilities.

It is not our aim to survey the whole field of transplantations in invertebrates and lower vertebrate classes as such, but to use these experiments merely as a means for the study of the organismal differences in their function of sustaining the tissue and organ equilibrium, and making thereby possible the maintenance of the individual organism. It is especially the experiments on coelenterates and planarians of Jacques Loeb, T. H. and L. V. Morgan, Wetzel, Peebles, H. D. King, E. N. Browne, Rand, Issayew, Child, Goetsch, Burt, Mutz and Santos on which our conclusions are based.

A. *Organismal Differentials and Organ and Tissue Equilibria in Coelenterates*

In a general way it can be stated that two sets of factors determine in coelenterates the kind of organ which is to be formed and its localization, namely (1) a more or less rudimentary preformed differentiation of the various parts of the body of an organism, and (2) the ability of parts of the organism to undergo structural changes and to restitute a whole organism from parts under varied conditions of the inner or outer environment. Instead of the relative fixity in the structural relations between the various tissues and organs which is characteristic of higher organisms, we find here a primitive and very incomplete differentiation, associated with a great degree of plasticity in the modes of response to altered conditions. It seems that each part of the organism has a tendency to produce a certain area of the organism or a certain organ system (pole) rather than another one, but often this tendency can be overcome; moreover, the readiness with which organs, other than those normally occurring in a given area, can be induced to form by experimental means (heteromorphosis) differs in different parts of the organism, the resistance being greater in those areas where the preformed organization, rudimentary though it is, tends to the formation of a more or less well differentiated organ area.

the organism which are not yet fully differentiated, to differentiate in such a way that the dominating directing organ is supplemented and that a complete organism develops. There are indications that the greatest potency of a certain part is required if it is to function as an organizer and this is an attribute mainly of the head, while apparently less potency is required for the regenerative, integrative function of inducing supplementary organ formation and of attaining in this way the formation of a whole organism by regenerative means. Those parts which are functionally and structurally indifferent, such as the middle piece, cannot act as organizers, nor can they induce regeneration.

The potential growth energy which is present in so marked a degree in these primitive organisms, as exemplified in their response to inductive regenerative influences and to organizer action, lies dormant in the normal organism; the mechanisms which cause induction of complementary parts and inhibit formation of similar parts are not ordinarily manifest. At each point the normal contact mechanisms are active and keep the various parts in a quiescent state; but as soon as (through a cut or otherwise) this normal action of contact substances is disturbed, local growth processes set in, which are determined in their character by a localized rudimentary differentiation, by regenerative and integrative processes, such as induction and inhibition at a distance, by organizer effects, and by environmental factors, all interacting with each other. This interaction leads to the establishment of a new equilibrium which takes the place of the previous disturbed equilibrium, and the most stable equilibrium is reached when complete individuals are integrated. Within these individuals the component parts are again equilibrized.

However, a wound not only disturbs the regulating and inhibiting influences which originate in the remaining parts of the organism and which would normally act on the wounded area, but it exerts also a direct stimulating effect on the tissues thus affected and its influence seems to extend even over a relatively great distance, accelerating the formation of a hydranth on removal of the inhibition existing normally. Through wound stimulation the organism or parts of it are transformed in such a way that they resemble, in their behavior and reactions, organisms during the budding, reproductive state, when they are very plastic and possess a greater growth momentum. We find here a condition analogous to the autogenous regulating mechanisms which determine tissue equilibrium also in higher organisms and the kind of disturbance in this equilibrium which follows injury. However, the relative importance of the various factors which become potent following the making of a wound cannot be exactly determined at the present time.

Predifferentiation and coordinated integrative actions not only manifest themselves through mechanisms which cause completion of incomplete organisms, but there must exist in addition, mechanisms which lead to degeneration of excess tissues or organs; we have referred to the resorption of small parts which do not possess a pronounced differentiation. There may occur also a coalescence of two small partners to form a single organism.

We may then conclude from these data that what corresponds to the fixed organ differentials of higher organisms, is, in the coelenterates, still in a very

latter extend their sphere of influence to distant parts, perhaps through diffusion of contact substances into distant areas of the body. Thus a well differentiated organ may be able to force a distant part of a transplant to form a heteromorphic organ, counter to its normal rudimentary differentiation. In this case, the size of the transplant becomes one of the determining factors. If the transplant exceeds a certain size, then its rudimentary preformed differentiation is able to control the regenerative, heteromorphic tendency of the host organ. Such a predifferentiation in the transplant may determine the mode in which the whole organism shall be formed, its integrative action inducing the formation of oral and aboral organs in accordance with the structure of the transplant, which may thus dominate over the integrative tendencies in the host. If a small piece is transplanted, there is evidently not enough material present to allow its predifferentiation to assert itself, because its opposite poles are very near to each other; the host, which is the larger partner, then dominates and apparently induces degenerative processes in the transplant, leading to its absorption. Perhaps the amount of active substance produced by short pieces is too small to overcome the opposing tendencies inherent in a larger piece.

A lack of a sufficient degree of predifferentiation in the transplant may be the reason why, under certain conditions, it cannot maintain itself in competition with the host and, instead, is absorbed by the latter. This applies especially to pieces from the middle zone; and, correspondingly, the middle zone of the host, by not inducing differentiation in a transplant, may lead to its absorption. On the other hand, if, as a result of the combined organizer and regenerative action, a part of an organism has been duplicated, the restitutive tendency can lead to a separation of the duplications, which may be followed by the formation of two independent organisms.

The tendency to supplement by regeneration a part of an organism in such a way that a whole predifferentiated organism develops has a counterpart in the tendency to form a normal whole from an organism, in which, through transplantation, a surplus of certain organs, for instance, tentacles, has been produced. The disequilibrium thus induced leads either to certain degenerative processes, presumably agglutination and reduction, or it may act as a stimulus to the production of certain organs, an effect which indirectly brings about the loss of excessive parts. The predifferentiated organism represents an equilibrated system, and disturbances in this system initiate various reactions aiming at the restitution of its equilibrium. It is remarkable how varied and different the mechanisms are which in the end all lead to the same result, the integrative newformation of an equilibrated whole.

In the experiments on which these conclusions are based transplants influenced the host and induced in it the newformation of organs, thus acting as organizers, or in other cases the transplants influenced the restitutive, regenerative activities of the host by actions at a distance; conversely, the host influenced, under certain conditions, also the regenerative or restitutive activity of the transplant. In these instances, as stated, certain organs, usually the more differentiated ones, are dominant over others and force those parts of

factor, such as a pull, can produce separation. Furthermore, nerve stimulation may fail to be transmitted from one partner to the other. This incompatibility between adjoining surfaces is also evident in the experiments of Peebles in Hydractinia. When a piece of Pennaria was grafted on Tubularia, the coenosarc united temporarily, but no union of the perisarc took place, and after formation of the hydranths the pieces disintegrated. Similarly, the union between Eudendrium and Pennaria was only imperfect and temporary. While here homoiotransplantation may, at least in some cases, be perfect, in heterotransplantation the union of the coenosarc does not last, and if farther distant species are used, injurious effects become still more noticeable.

In general, buds develop, as H. D. King has shown, at the point of union of two different kinds of organisms; these represent a mixture of the constituents of both partners and thus constitute a chimaera. When the organismal differentials of the two partners are markedly similar, the mixture is more complete and the character of the tissue interaction differs from that seen when the organismal differentials have less similarity. In the latter instance parts of one organism have a tendency to penetrate as a connected mass into the other, the constituents of both partners remaining more separate and distinct than when the organismal differentials are very much alike. In a very interesting way the domination of one organism over the other, when they differ in the constitution of their organismal differentials, has been shown in the experiments of Goetsch and Issayew, who found that when two individuals belonging to different species are united into one organism, budding often takes place, the buds representing chimaerae of various kinds in which, however, the constituents from one of the two species predominate. Issayew obtained chimaerae also by cutting individuals from two different species into small particles, which, when mixed, united to form one complete organism representing a mosaic of both partners. The union of Pelmatohydra oligactis and Hydra vulgaris into a chimaera leads to a struggle between the constituents of the two partners, in which the former gradually infiltrates and almost replaces the latter; Pelmatohydra dominates and apparently only certain interstitial cells of Hydra vulgaris remain preserved. The buds from such chimaerae may be either Pelmatohydra or a mixture of both species. The remaining interstitial cells of Hydra vulgaris are totipotent and may give rise to whole organisms.

The dominance of one species over another in heterotransplantation may also become manifest in another way. If an excess of tentacles has been produced as a result of transplantations, the dominant species may determine the number of tentacles which shall be absorbed and, in the end, the number which is characteristic of the dominant species remains. We see that even in this case of heterotransplantation the integrative factors, tending towards the reestablishment of an organ equilibrium which accords with the predifferentiation of the dominant species, are active. As a rule, that species which, in the separate state is the more vigorous one dominates. We shall find also in amphibians joined together in embryonal stages, a dominance of one partner over the other, in accordance with the more rapid growth and greater size of one of these species in the free-living stage.

plastic, modifiable condition. It is due to this plasticity of organ differentials and to the readiness with which transformations and newformations of organ systems and parts of the body take place that individuals are restituted from parts. But this factor alone would not insure the ready restitution of individuality. There must be added to it a certain autogenous state in which organ systems interact perfectly in such a manner that a relatively stable equilibrium is maintained. Some of the mechanisms which participate in the maintenance of this equilibrium we have analyzed in the preceding pages. Any disturbance in this autogenous equilibrium, consisting in the balancing of these organ systems, activates mechanisms which result in the integration of the organism. It is the relative lack of fixity in organ differentials, their ability to change within certain limits, that make possible the integrative activity of the mechanisms leading to the restoration of the individual.

So far, we have analyzed the interaction of organs and organ differentials and their significance in the maintenance of the equilibrium which makes possible the integration of parts into the individual organism. We shall now compare with the nature of this equilibrium, the mode of action of the organismal differentials in this class of animals. Here we notice that the plasticity of the organ differentials is somehow bound up with a relative lack of fixity of the organismal differentials, or at least of the effects which differences in organismal differentials would induce in higher organisms.

We find, accordingly, that in Hydra auto- and homoiotransplantations seem to succeed equally well; similarly, there seems to be no difference in the results when several autogenous or homoiogenous pieces are joined together, the integrative as well as the organizer impulses being transmitted in a normal manner from one piece to the other. At the point of union corresponding tissues of homoiotransplants and host may unite perfectly in Hydra, without any scar remaining visible. Homoiotransplantation in Hydra succeeds very well, even if the two partners have been made unequal in their contents in algae. However, we must not necessarily conclude from these experiments that homoiodifferentials do not exist in these organisms. While this may perhaps be the case, there still remains the possibility that they do exist in a rudimentary form, but that they are not strongly enough developed to lead to noticeable reactions and that the tissues have a power of resistance sufficient to overcome unfavorable conditions caused by a difference in organismal differentials. With this conclusion harmonizes also the observation that while in organisms like Tubularia homoiotransplantation may apparently be perfect, yet in some cases separation between host and transplant takes place after a time.

The results of heterotransplantation differ noticeably from those of homoiotransplantation. Even if the transplantation succeeds, the differences in race or species differentials may cause the union to take place more slowly and the resulting combination may only be temporary, separation occurring perhaps at a later date. On the other hand, it seems that union of different species may permanently succeed in certain instances. In Hydra the union of heterografts may, however, not be so firm as that of homoiografts; the surface of contact between the species may at first become smaller, until at last some mechanical

restitution of a head in the headless part, although also in this type of heterotransplantation certain disturbances appear; thus, distance actions which take place in cases of homoiotransplantation, leading to reversal of polarity (heteromorphosis) in the transplant or to the formation of buds in the host under the influence of the transplant, do not occur. There is, therefore, under these conditions an interference with the transmission of the regenerative or organizer influence, which under other circumstances would have passed from host to partner, or vice versa. For the most part, either an absorption of the transplant takes place in these heterotransplantations, or the grafted head separates from the host. We may then conclude that after heterotransplantation incompatibilities develop between the partners or between a transplanted organ and the host at the point of union. This often leads to early separation and, in addition, difficulties may possibly develop in the passage of active substances from one organism into the other.

We must now inquire how far the reactions which have been observed when we unite organisms belonging to different species or races in hydrozoa, can be considered as due to differences in organismal differentials. There are two circumstances which favor this interpretation: (1) The severity of these incompatibilities corresponds approximately to the distance of relationship between the parts which are joined together, and (2) the reactions after heterotransplantation seem to occur irrespective of the place where the two strange organisms are united; this fact suggests the presence of the same organismal differential in all parts of the same individual.

As stated above, while in general only heterodifferentials lead to noticeable incompatibilities in hydrozoa, we cannot therefore conclude that individuality differentials do not exist in these primitive forms. We must consider the possibility that each individual within a race or species has its own individuality differential, which differs from that of every other individual, but that the incompatibilities which result from these differences between differentials in the lower types of animals are too slight, in proportion to the resistance of the affected tissues, for injurious agencies to become manifest. It is this relation between the degree of incompatibility, the resulting injurious reaction on the one hand, and the resistance of the transplant, which might be expected to determine the degree of disequilibrium arising from differences in the differentials. If the individuality differentials are as yet only very slightly developed, the incompatibility resulting from the union of parts of different individuals may not become evident. On the other hand, it is possible, after all, that individuality differentials are not yet present in these primitive organisms. The second alternative might even be the more probable one, because there is reason for assuming that in young vertebrate embryos fully developed individuality differentials do not as yet exist. By analogy we may extend this conclusion also to adult individuals belonging to very primitive vertebrates.

We must now return to a discussion of the conditions which maintain the normal organism in a definite formative and functional equilibrium, and of the similarities or the differences observed between higher and lower organisms in this respect. In higher organisms such a formative equilibrium depends, in part at least, on local conditions affecting the tissues; interactions

While thus the difference in race- and speciesdifferentials in the pieces joined together may lead to antagonistic actions between the tissue constituents of the different grafts, yet to a certain extent it is possible for the tissues in such buds and chimaerae to live and grow side by side without the manifestation of a hostile reaction. Somewhat comparable results can be obtained, as we shall see later, in the transplantation of regenerative buds of extremities in amphibia, in the ingrowing of sidelines from one partner into the other in heterotransplanted amphiban larvae, or, as we have mentioned already, in the ingrowth of a nerve from one partner into the other in parabiosis in rats. In all of these conditions there is a lack of manifest reaction on the part of tissues which are in close contact with each other, although they differ in their organismal differentials. In such cases we have to deal either with ontogenetically or phylogenetically very primitive forms, or with regenerating tissue which does not yet possess the fully developed organismal differentials, or at least the mechanism of reaction against such differentials. In the case of parabiosis in rats we may have to deal with relatively slight differences in organismal differentials.

In accordance with the experiments mentioned above, Mutz found that pieces of Hydra and Pelmatohydra can be joined together in the long axis of the body, the different constituents retaining the character of their own species. However, the growing together takes place with much greater difficulty than in homoiotransplantations and for a long time the place of union remains visible; but in the end a uniform, apparently normal Hydra, though in reality representing a chimaera, may result from this transplantation. On the other hand, the green Chlorohydra cannot be joined to the brown Pelmatohydra, to Hydra vulgaris or Hydra attenuata, separation taking place within eight days. It seems that in this case the presence of algae in Chlorohydra intensifies the difficulties of heterotransplantation. While in the case of homoiotransplantation algae do not interfere seriously with the result, this is not so if distinct races or species of Hydra are combined; then, the presence of Algae increases the incompatibility between the partners, as the experiments of Goetsch have shown. But even if the number of algae is approximately the same in different partners, there still remains noticeable the difference in race or species constitution. If the head of Hydra vulgaris or attenuata is transplanted to Pelmatohydra, the union between transplant and host is only a temporary one, lasting usually from three to five days, or, at most, two weeks. In this instance, the transplant is not able to act as readily as an organizer, inducing a head formation in the host, as it would have been if the transplant and host had been homoiogenous. There forms, instead, at first a bridge of tissue, growing from the host in the direction towards the transplant. This bridge represents a somewhat indifferent kind of tissue in which tentacles are lacking; but after separation of the transplanted head and host has taken place, it may develop in some cases into a small head, while in others it is drawn into the host and absorbed. The organizer action is therefore interfered with in such a heterotransplantation. However, when reciprocal organismal transplantations of pieces of Pelmatohydra and Hydra attenuata are made, the two pieces may remain united long enough to make possible the

equilibrium may, in certain instances, in hydroids as well as in planarians, lead to the separation of autogenous parts of an individual which have been present in excess, and which have become superfluous. Here it is evident that regulatory, integrative mechanisms of an unknown nature constitute the primary process, and this is followed by the creation of a wound surface as a secondary effect; the latter is, therefore, caused by the action of these integrative mechanisms; (4) there are indications that the mechanisms mediating the maintenance of an individual as an equilibrated system are not so well transmitted at the points of union between heterogenous partners. We may expect in this case regulatory processes to be set in motion, leading to attempts at new integrations of individuals, with the resulting separation of the incompatible parts.

We have seen that an individual hydrozoon can be divided, and that each part can give origin to a complete organism. The degree to which divisibility can be carried depends on whether such particles are kept in their normal medium free from contact with other individuals, or whether they are transplanted to another organism; if transplanted, the antagonistic influences which the host may exert on the transplant may make it necessary for the transplant to have a minimum optimal size before it is able to restitute the whole. We may again refer in this connection to the experiments of Issayew, which have shown that particles from different individuals may be joined together in such a way that they form a whole organism, which represents the mosaic of a chimaera. In this case each particle forms part of one whole, at the same time still retaining its own organismal differential.

In hydrozoa, as has been noted, the tendency exists to produce single individuals through processes of coalescence, as well as of splitting leading to supplementary newformations. Correspondingly, stolons of hydroids belonging to the same colony, and even stolons from adjoining colonies, may coalesce. Also, larvae of coelenterates may unite among themselves and give origin to a new colony, or they may join a part of an already existing colony and help to enlarge it. However, the mechanism which usually leads to colony formation is that of budding. The question may be raised whether it is the individual polyp or the colony of polyps which shall be considered as the bearer of the individuality. As far as the individuality differentials are concerned, we may assume that, provided they exist at all, they are the same in all component parts of a colony. Even strange colonies belonging to the same species may have identical individuality differentials if they have developed from buds given off by the same colony. But differences in conditions analogous to individuality differentials in such colonies might possibly have originated under the influence of different environmental factors, which were able to modify the different colonies; furthermore, it is conceivable that somatic mutations might occur and lead to such changes. In higher individuals, differences in individuality differentials are, as a rule, due to processes which take place during the formation of the germ cells and during fertilization. We may therefore expect less sharp differences in the nature of the individuality differentials in organisms propagating by

take place between adjoining tissues which are of a regulatory character and keep the animal equilibrated. However, distance substances, in the form of hormones, may also participate in this equilibrium as secondary factors, though their action is less important and more specialized. In the case of hydrozoa, conditions are in some essential respects similar to those of higher organisms; the equilibrium depends on local and distant factors and it can be disturbed through local as well as through distant changes; also, there is reason for assuming that in both instances definite substances mediate these effects. Furthermore, in these primitive organisms the organismal differentials, as well as what corresponds to organ and tissue differentials in higher organisms, participate in the maintenance of an equilibrium; but the particular structure and function of adjoining autogenous parts of the organisms seem to be better able to induce growth processes of various kinds in hydrozoa than in higher organisms, on account of the greater plasticity of the tissues and organs in the former. Important also in these lower forms are specific distance substances, which, acting in a stimulating or an inhibiting manner, are able to modify the structure of the organism. There is a third difference between higher organisms and hydrozoa in the stabilization of the equilibrium. In higher organisms the equilibria depend upon the local interaction of tissues bearing the same individuality differential and they are therefore essentially autogenous in character. In hydrozoa, on the contrary, parts of an organism differing in the character of their individuality differentials may, in general, substitute for each other; incompatibilities, with resulting disturbance of the formative equilibria, as a rule become manifest only if distinct differences in species differentials exist between adjoining tissues. We cannot therefore consider this equilibrium in lower forms as strictly autogenous in character; it is of a homoiogenous, as well as of an autogenous nature.

The next problem to be considered concerns the incompatibilities and disturbances of equilibrium which may take place, in organisms bearing heterogenous differentials, after a primary union and an apparently complete formative equilibrium have been established. These changes may be due to two different causes: (1) The primary incompatibility of the organismal differentials may gradually increase, the resulting disturbance of the equilibrium becoming manifest in the appearance of growth processes in whole organisms, which otherwise would occur only in isolated parts; or (2) the original incompatibility of the organismal differentials may lead to a primary separation of the adjoining surfaces of the heterogenous parts and this process may be followed by regenerative changes. We believe that the first interpretation is the more probable one for several reasons: (1) In certain cases, when the union between adjoining homoiogenous pieces was apparently perfect, secondarily a separation also took place. Presumably a formative change at the point of union was here the primary process, which was followed by separation; (2) the separation may occur in some classes of animals not exactly at the junction of the two surfaces, but at a neighboring point. This indicates that either growth processes or changes of a degenerative character induced the separation; (3) one of the mechanisms which help to reestablish a stable

complete organism. In the latter case there must be a local factor active, which causes the various organs to reproduce each its own kind, although even under these conditions inducting factors acting in the direction of the long axis may play a certain part. Likewise the fact observed by Child, that even in the absence of a head an isolated piece of Planaria is able to regenerate all parts representing the levels posterior to its situation in the organism, points to the existence of a predifferentiation in these parts, and there are some indications that it is the nervous system which plays, here, an important role in determining the rudimentary differentiation.

In Planaria, as in a similar manner in hydrozoa, it is possible to demonstrate the existence of organizers. In both classes of organisms it is especially the most differentiated organ area, the anterior pole or head, which not only dominates the structure of the organism, but may also act as organizer (Child, Goetsch, Santos). Furthermore, both classes show the same types of induction, and the inducting organ gives origin to its own kind of organ in the material acted upon; in hydrozoa a transplanted head gives rise to a new head, and in Planaria, according to Gebhardt, the eyes of the host may induce eye formation in a bud from the posterior part, which has been transplanted into the head region. In addition, a second type of induction has been established in Planaria, especially by Moretti, Goetsch and Santos. Goetsch observed that a transplanted head can induce in the host a reorganization, which leads to the development of a postcephalic region. Santos grafted a piece from the ganglion region of Planaria into the prepharyngeal levels of the host. If the transplant was of a sufficient size, it gave rise to a head and determined in the host a postcephalic outgrowth. If implanted into postpharyngeal levels of the host, the transplant not only determined postcephalic outgrowth in the host, but, besides, it caused a further reorganization, with the development of a pharynx and postpharyngeal region. But the reorganizing influence of the grafted piece extended in the host also in an anterior direction and in this way it could determine a reversal of the polarity. However, the host too may exert an influence on the grafted part. This was indicated by the fact that when the union between host and transplant was complete, the host inhibited the perfect development of a head from the graft, while an incomplete union gave the transplant a chance to develop in accordance with its own potentialities.

As Rand has found, the inhibiting influence which a graft exerts on a wound, in a more or less specialized region in the host, varies somehow in an inverse relation to the distance of the inhibiting material from the wound surface. This suggests that we may have to deal with a diffusible contact substance, which decreases in concentration with the distance between graft and wound. Besides such inhibiting effects we have then to deal here, as in coelenterates, with two kinds of actions. One leads to the reproduction of the same organ as that which acts as an organizer and the second represents a complementary, integrative mechanism, which causes the completion of a whole organism from a part.

We may assume that the inducting action of the transplanted head region

asexual budding or having the power to restitute the whole organism from a part, than in those propagating only by sexual mechanisms.

If we consider individuality from the functional point of view and attribute it to an organism able to live and function independently as an equilibrated mechanism, to which different parts of the whole contribute in a distinctive manner, then we can attribute individuality to the single hydrozoon as well as to a colony, in which certain constituent parts may exert different functions. However, this seems to be a problem of minor importance, because the term "individuality" is not rigidly defined; it is used in reference to organisms merely in order to describe certain of their characteristics. On the other hand, it may be worth while to inquire whether a connection exists between the lack of a manifestation of finer differentials in these primitive organisms and their great plasticity, as exemplified in their readiness to form organs under the influence of external and internal environmental factors and propagate asexually. All the evidence tends to the conclusion that such a connection does exist, although the underlying mechanism is not yet understood. It is presumably also these latter characteristics which provide such organisms with the potentiality of immortal life, which higher organisms no longer possess; in the higher organisms at best, certain tissues and cells may possess such a potential immortality.

B. *Transplantation and Individuality in Planarians*

In many respects conditions in planarians are very similar to those found in hydrozoa. In planarians we have also to deal with a very plastic living substance in which, however, again a definite state of rudimentary preformed differentiation exists; within a certain range it is possible to change the polarity of organs and thus to produce heteromorphosis. However, there are indications that in proportion to the greater differentiation which exists in planarians external factors do not quite, to the same extent, influence organ formation and change the polarity in these organisms as they do in Hydra. In order to evaluate the relation of organ differentials and of the equilibrium between the parts of an organism, on which its existence as an individual depends, to organismal differentials, we shall also in this instance first discuss very briefly the factors that determine polarity, fixity and transformability of parts of the body and its various organ systems.

The existence of a predifferentiation in this class of animals is indicated by the fact that the anterior pole has a greater head-forming tendency than the posterior pole. Thus, while the posterior (aboral) pole has the power to regenerate a head, its ability to do so is less than that of the anterior (oral) pole. Furthermore, in the region where the sex organs form, proliferation in the host tissue may, according to Gebhardt, readily lead to the casting off or resorption of the transplant. The existence of a rudimentary differentiation comes out also in the specific inhibition in organ formation; thus, the proximity of a head inhibits head formation (Rand, Goldsmith) and that of a tail inhibits tail formation (Rand). It is also indicated by the fact that if Planaria is split lengthwise into halves, each half may regenerate into a

latter, which have to be considered as possible factors responsible for the regenerative and integrative changes setting in after an injury.

Homoiogenous tissues can bring about such an equilibration, whereas after transplantation of pieces belonging to a different species or genus, this result is only temporary and after some time regenerative, integrative activity sets in at the point of union, so that, as Goetsch observed, the two parts of two organisms previously joined together separate again. This fact makes it reasonable to assume that contact substances whose organismal differentials are not too distant are needed for equilibration. Also, the inductive action, which a transplant exerts on the mesenchymatous tissue of the host, takes place, according to Gebhardt, only if transplant and host are homoiogenous; if the adjoining tissues carry heterodifferentials no induction is noted. On the other hand, the organizing action of ganglionic material, studied by Santos, is effective not only when both graft and host belong to the same species, but also when they belong to different species; when the graft belongs, for instance, to Planaria dorotocephala and the host to Planaria maculata, or vice versa.

As in hydroids, so also in planarians homoiotransplantations succeed readily and distinct differences between the effects of auto- and of homoiotransplantation have not been established; we have therefore no indications of the existence of distinctive homoiodifferentials, though the existence of heterodifferentials has been definitely demonstrated. As stated, in certain favorable cases heterotransplants act like homoiotransplants; but it seems, as a rule, that contact and distance mechanisms active at the point of union between two homoiogenous pieces are ineffective in heterogenous combinations. Heterotransplantation of small pieces seems not to lead to a complete union of the graft with the surrounding host tissue.

So far as the plasticity of organs and the lack of manifestation of finer organismal differentials are concerned, there is thus a fargoing likeness in hydroids and planarians, and the same general conclusions apply in these two groups of animals as to the similarity of the mechanisms which maintain the intraorganismal equilibrium and the absence of distinctions between autogenous and homoiogenous tissues in this equilibrium. Various types of organizer and regenerative, integrative influences play a role in determining the mutual relations of organs and tissues in these lower organisms, all tending to reestablish the original equilibrium when it is disturbed. It is this autogenous equilibrium, as we have studied it in higher organisms and as it exists in a wider sense also in these primitive organisms, which determines the maintenance of individuality. However, here the type of interactions between organs and tissues which helps to sustain the normal equilibrium, or to reestablish a disturbed equilibrium, is in some respects more accessible to analysis than are the corresponding mechanisms in the higher organisms. In the latter, accompanying the greater refinement in individuality, the means of restoring an unbalanced equilibrium, in the sense in which it can be accomplished in these very primitive organisms, are lacking.

which Moretti, Goetsch and Santos observed, is effective also in the normal organism and here helps to maintain its polarity, a conception emphasized especially by Child in his axial gradient theory. There are two mechanisms through which such an effect could be accomplished: (1) Through contact action the dominant, most differentiated part could transmit an inductive effect to the adjoining posterior part and this part in sequence could exert a similar inductive effect on the nearest adjoining region in the antero-posterior direction; in this way a distance action could be brought about, in which the inductive effect becomes gradually weaker; or (2) substances could be produced by the dominant part, from which they pass to neighboring and to distant regions in gradually decreasing concentration. But there is reason for assuming that such actions do not affect an indifferent material, but one which, while plastic within a definite range, still possesses a certain pre-differentiation which varies in different parts in fixity.

It is possible that parts, other than the dominant part, also exert an inductive distance effect. Thus L. V. Morgan has shown that when a head is cut off in Planaria and a small piece is placed on the wound in an inverted direction, so that the oral pole of the graft joins the oral pole of the host, a distance action is exerted by the larger piece, representing the host, which causes the aboral pole of the transplant to form a head. Such an interpretation of the experimental findings would apply if we assume that the head formation in the transplant was not the result of unknown external factors, but was caused by an integrative type of induction.

As in the case of hydroids, so also in planarians, the mechanisms underlying the change of abnormal structures to a normal organism may lead to the absorption of excess organs. In other cases the mechanism underlying integration may lead to a duplication of an organism, as when two complete organisms form following the production of two heads and two tails through partial lengthwise incisions in the anterior and posterior parts.

According to Goetsch, a transformation of one organ into another, a coalescence of two organs, or a newformation of an organ, takes place by means of an intermediate stage, in which, at first, under the influence of various stimuli, an indifferent tissue develops, which secondarily undergoes the specific differentiation. Fully differentiated organs cannot be directly transformed. The less marked the differentiation is in a certain organism, the less fargoing need be the preliminary changes, as well as the later differentiations, which make possible the transformation of one organ into a different one. In hydroids the regeneration followiog removal of a part of an organism can be prevented by grafting another piece of a hydroid on the cut surface. In the same way it is possible to prevent regenerative action also in Planaria.

The facts to which we have referred indicate that the normal Planaria represents an equilibrated system in which there are various mechanisms of induction acting on the neighboring tissue, as well as at a distance. It is the removal of these influences, as well as the direct effect of a new medium surrounding the injured tissue and the altered mechanical conditions in the

A second problem prominent in the earlier experiments concerned the possibility of changing species characters of parts of organisms by means of heterotransplantation. Is the host able to impress his own organismal differential on the transplant? We now know that species, as well as individuality differentials are gene derivatives and are therefore essentially fixed, although their manifestations may be modifiable within certain limits.

If we now consider the investigations in lumbricidae which have a bearing on the problems with which we are especially concerned, namely, the evolution of the organismal differentials and their relation to the degree of plasticity of the organism in its response to environmental changes, it has been shown that, as a general rule, homoiotransplantation of pieces which are viable succeeds readily, host and transplant remaining permanently united. Following transplantation, a union of the corresponding organs, such as integument, vessels, intestines and nerve strands, takes place, and movements as well as mitotic divisions in the tissues play a role in this process; thus one harmonious individual is produced in which the organ systems derived from different individuals function well, and it is only by means of differences in pigmentation that the homoiogenous constituents of such individuals can, in some cases, be distinguished. It seems that especially the union of the nerves of the two partners is important in homoio- as well as in autotransplantation; if the nerves do not properly unite, then regeneration may occur at the point of junction of the pieces and a new head may grow out, or the partners separate, even if outwardly the union between the partners has been perfect. Apparently the nerves play, here, an important part in determining regeneration, and we may recall the fact that also in Planaria Santos found indications that the cephalic ganglia may determine head formation. It is apparently the contact with corresponding living nerve tissue which keeps the nerves in a quiescent state, preventing their regenerative outgrowth and thus their stimulating effect on the growth of other surrounding tissues. However, the evidence as to the significance of nerve tissue in regenerative and integrative growth processes, especially in cells in planarians, is still contradictory, and in lumbricidae even a defect in the union of the body walls of the two pieces may lead to a newformation of a head, irrespective of the presence of nerve fibers.

While, then, in this class of animals there are apparently no differences between auto- and homoiotransplantation, yet by means of certain experimental procedures it is possible to bring out such differences; thus, if three pieces are joined together, the middle piece being inserted in an inverse direction, this combination remains alive permanently only in autotransplantations, while in homoiotransplantations some difficulties appear. But, there remains the possibility that the superiority of autotransplantation may be due to the fact that in this instance the pieces fit together better mechanically and that individuality differentials are not concerned in this result. Similar observations were made in Hydra by H. D. King in joining together more than two homoiogenous pieces.

In lumbricidae, the differentiation of the organism is farther advanced

Chapter 2

Transplantation and Individuality in Higher Invertebrates and in Amphibia

IN THIS CHAPTER we shall analyze individuality first in lumbricidae, which represent a transitional form between the very primitive invertebrates, already discussed, and the more differentiated echinoderms and arthropods. These latter will then be considered, and lastly, amphibia, as representing a less highly developed type of vertebrate.

A. *Transplantation and Individuality in Annelids*

The lumbricidae differ from the planarians in a considerably greater fixity of their organs and presumably in a correspondingly greater specificity and fixity of the substances on which the differences between organs depend (organ differentials). While the organs have not yet become entirely rigid, still the differentiation between head and tail parts is more fixed than in planarians. In accordance with this change in the organs we find a greater differentiation in the organismal differentials, as is indicated in the transplantation experiments on lumbricidae which have been carried out especially by Korschelt and his associates, Joest, Rutloff, Leypoldt, Harms, Rabes, and more recently by Mutscheller. The earlier of these experiments antedated the majority of the investigations on coelenterates and planarians. At that time attention was focused on problems which have since receded into the background. Thus the problem as to the significance of a reversion of polarity in transplantation, which had been introduced largely through the investigations of Voechting in plants, dominated research to a large extent at this earlier period, and even much later we find Schoene studying polarity in transplantation of vertebrate skin.

As to polarity, two questions might be asked: (1) Is there inherent in these organisms an orientation of their constituent parts comparable to the organization of a magnet, and is it therefore necessary that the transplant be inserted into the host in a definite direction if transplant and host are to be mutually compatible? As far as is known, this does not hold good in the animal series. (2) Do the actions of contact substances and of distant substances exert different influences on regenerative processes, and in particular on wound healing, in a normal and a reversed orientation of the transplanted piece? This may be the case in the more primitive organisms, where regenerative processes of an integrative character play a much greater role than in the higher organisms, and where the organ differentials are not yet so rigid as to prevent heteromorphosis. However, in some instances certain subsidiary factors may differ at the two poles of a transplant and then such factors may play a role also in higher organisms.

in a loss of certain structures, followed by the formation of new structures, or, in some instances, in the direct transformation of organs, without the previous loss of other structures. These observations point to the existence of a somewhat furthergoing plasticity in the structure of annelids, which permits the transformation of regenerating abdominal segments into segments with the character of thoracic segments. On the other hand, experimentally produced duplications of considerable size may persist unchanged under conditions in which, in the more primitive and plastic organisms, various regulative mechanisms would have eventuated in the formation of normal individuals. As far as the organismal differentials are concerned, homoiogenous combinations are possible in lumbricidae, without leading to disharmonies which, as we have seen, take place in parabiotic partners in mammals, owing to the greater refinement of organismal differentials in the latter.

We see, then, that in general the differentiation and fixity of the organism is much more advanced in lumbricidae than in hydroids and planarians; correspondingly, the inductive distant action has decreased in effectiveness in the former, and it is likewise due to their relative fixity in organization that an organizer action, in which a differentiated part grafted on a host induces here the development of its own kind of an organ, seems not to have been observed in this class of animals. Such an organizer would probably be unable to act effectively with this less plastic material. On the other hand, there is reason for assuming that the second kind of inductive action leading to integrative regeneration is still, though to a much diminished extent, potent even in lumbricidae.

Heterotransplantation succeeds in lumbricidae with much greater difficulty than homoiotransplantation. In the large majority of cases heterogenous pieces remain united only for a few days, then separate or degenerate. In other cases there may be a better union by means of scar tissue covered by epithelium; secondarily, muscle, nerves and vessels may grow through it and into the heterogenous tissue. Thus also in heterotransplantation blood vessels as well as other organs may unite with the corresponding organs of the partner and a common circulation be established. But even under these conditions while there is, at first, an apparently perfect union of the two heterogenous pieces, a separation may take place subsequently and as late as after five weeks. As a result of changes occurring at or near the place of union, the latter becomes looser and a mere mechanical pull may readily separate the two partners. However, in one instance Korschelt succeeded in keeping a combination of Lumbricus rubellus and Allophora terrestris alive for a period of eight to nine months. In pieces thus temporarily united, in which a smaller heterogenous piece has been grafted in an inverse direction on a larger host whose head has been cut off, the host may induce the beginning formation of a heteromorphic head in the smaller anteriorly situated graft; but usually at the point of union a new head develops and then the pieces separate. The heterogenous contact substances, or, more generally expressed, contact mechanisms, which are active at the cut surfaces do not keep the adjoining parts in an equilibrated, quiescent state and can not therefore prevent regen-

than in planarians and the relative importance of inductive factors, acting by means of distance substances given off by transplanted pieces, is diminished as compared with the more primitive organisms, although it is not yet entirely lost. Likewise, the action of external environmental factors on organ formation is not evident in lumbricidae in the sense in which it exists in the case of hydroids. Korschelt and Mutscheller have shown that the ability to form a head is limited to the most anterior part of the body, and that the farther back the segments are, the greater is their tendency to form a tail. The gradient in organization in Lumbricus is thus quite definite. If one transplants to the anterior pole of an animal, whose head has been cut off, a posterior part (tail) of a worm in the inverse direction and then cuts off the end segments of the transplanted tail, the inductive action of the large posterior piece, which would tend to call forth the production of a heteromorphic head at the end of the graft, cannot overcome the strong organ-specialization of the tail segments which have the inherent tendency to produce a tail. But if a still larger number of the posterior segments of the tail piece are removed before a remaining piece of the tail is grafted inversely on the anterior cut surface of the host, then the inductive, integrative action of the larger partner can overcome the less specialized organ differentials which exist in the graft, at a somewhat greater distance from the posterior end of the animal, and formation of a head *may* take place at the free cut surface. There exists, thus, a competitive struggle between the inductive action of substances or mechanisms, which act from a distance and which may tend to produce a heteromorphosis, and the *fixity* of the organ differentials in the transplant, which, when unopposed, would lead to the reproduction of a tail organ. In this struggle the larger partner has an advantage over the smaller, a condition applying similarly in hydroids and in planarians. A corresponding experiment which L. V. Morgan carried out in Planaria, leading to the heteromorphic development of a head at an aboral cut surface grafted inversely on the anterior cut surface of a larger piece, succeeded more readily, because in planarians the organ differentials are not yet fixed to the same degree as in lumbricidae. The more pronounced differentiation of organs in these latter animals diminishes the plasticity of the organism and the ready transformation of polar organs, as well as the importance of integrative induction.

More recent experiments of Julian Huxley and Gross with the polychaete worm, Sabella, indicate that the making of a wound as such may exert a stimulus which acts not only locally in an area adjoining the wound, but which may also act at a distance and influence the character of the structural changes which shall take place. Thus the cutting off of a regenerated head may not only influence a transformation of abdominal segments into thoracic segments near the head pole of the animal, but it may also cause, under certain conditions, a further transformation of regenerating abdominal segments situated at the tail pole of the animal into thoracic segments. Also, small lateral wounds may influence the changes which take place in adjoining, and even in more distantly situated segments; these changes may consist either

We may conclude from the principal facts relating to organ and organismal differentials in the relatively primitive classes which we have considered so far, that while with the progress in phylogenetic development the differentiation of the organs advances, their plasticity decreases and, correspondingly, the effectiveness of the inductive processes leading to the reestablishment of normal individuals is diminished; however, there is not a proportional advance noticeable in the differentiation of the organismal differentials, or at least in their manifestation. In all these classes heterotransplantation between nearly related species seems to succeed, although as a rule with greater difficulty than between homoiogenous organisms, while between farther distant species it does not succeed. But, it is possible that the greater regenerative power of the hydroids and also of the planarians, as compared with that possessed by lumbricidae,—a regenerative power which leads to integration of defective organisms,—may serve to cover up the fact that they actually do possess less differentiation of the organismal differentials than the lumbricidae and less sensitiveness to strange differentials. The greater regenerative power of the hydroids may lead to a more ready outgrowth from a cut surface and to subsequent separation of the partners in case the organismal differentials are not quite compatible, and this condition may make it appear as though the reactions against differences in organismal differentials were more severe in the more primitive organisms than they actually are.

B. *Transplantation and Individuality in Arthropods and Echinoderms*

In general, the regenerative power is very limited in insects and moths, especially after metamorphosis has taken place, and this condition interferes with transplantability to a certain extent. It also seems to be associated with an increase in the rigidity of organization.

In moths, experiments in transplantation were carried out by Crampton as early as 1899. At that time, as we stated in reference to experiments in lumbricidae, the problems of polarity and of the preservation of the species characters in transplant and host were prominent; also, the question as to the behavior of the various organs of host and transplant to each other had been introduced through the experiments of Born with embryos of amphibia. On the other hand, the problem as to the effects of variations in relationship between the partners, or between donor and host, on transplantation played an unimportant role at that time. In Crampton's experiments parts of pupae were used; on account of their low regenerative power only the skins or other organs situated near the surface, but not the internal organs, united after transplantation, and positive results were obtained therefore only in a minority of cases. While homoiotransplantation of small pieces of tissue was successful in a number of instances, heterotransplantation succeeded not at all, or at best only exceptionally. However, if instead of grafting small pieces of tissue, whole segments of the animals were transplanted, a procedure which does not exactly correspond to parabiosis because of the inability of the isolated segments to lead an independent existence, both homoio- and heterotransplantation succeeded, but the former apparently somewhat better than the

eration. The attachment of a heterotransplant to the adjoining host tissue is from the beginning less complete than it would be in case of homoiotransplantation and gradually degenerative changes occur in the transplant.

Similar are the results if, instead of larger heterogenous transplants, small pieces are grafted on defects in larger pieces. If small pieces of skin together with the adjoining muscle are thus heterotransplanted into wounds in lumbricidae, they remain as a rule preserved only for a certain time; but in exceptional cases the transplant may maintain itself apparently without change for as long as nine months. Leypoldt used in experiments of this nature, regenerating, heterogenous skin which was not yet fully developed; but in most instances it was either soon discarded or it was gradually absorbed through the activity of the adjoining tissue of the host. Even after the pieces had healed in, in an apparently perfect condition, subsequently degenerative changes took place in the transplants and led to their gradual absorption. On the other hand, homoiogenous pieces of skin usually were much more readily preserved for a long period of time, or even permanently. When ovaries were heterotransplanted, in a small minority of cases the grafts remained in good condition as long as for three months or even for one year, provided the species were nearly related; but if they were farther distant, the transplants were injured and were much more quickly absorbed. It is of interest that it was also possible to obtain heterofertilization of the eggs which developed in the heterotransplanted ovaries. The F_1 generation of such hybrids possessed characteristics of both parents, but they were sterile and soon died.

We may then conclude that heterotransplantation may succeed, although usually with some difficulty, in lumbricidae as well as in the more primitive hydroids and planarians, provided the species used are nearly related; otherwise the incompatibility of the organismal differentials leads to an early separation or destruction of the transplant.

Somewhat similar are the factors which are active in transplantation in the oligochaeta Criodilus, as we may conclude from the experiments of Tiara. In this organism certain kinds of heterotransplantation succeed, while others do not. We have here, likewise, a limited degree of organ differentiation, the anterior segment having the tendency to form a head, the posterior segment a tail; but as in lumbricidae, heteromorphosis may occur as a result of certain mechanisms acting in such a way as to force upon a smaller piece, from a distance, the formation of an organ contrary to the rudimentary differentiation existing in the smaller piece. Thus a larger sized partner may gain dominance over a smaller one.

In this case also, the nervous system may perhaps determine whether or not a head formation shall take place at the point of union between two pieces. A head forms at the anterior cut surface if the nerve strands of the partners do not unite. Under these conditions the regenerative activity of the cut nerve seems to furnish the stimulus for a head formation. That in these organisms a certain plasticity of organ formation still exists follows also from the fact that the epidermis of the adult animal has the power to regenerate the nervous system.

another individual of the same species; this kind of transplantation apparently succeeded also between different species if they were nearly related (Hydrophilus and Dytiscus), but not between farther distant species. Finkler states that the head thus transplanted determines to a large extent the sexual reflexes and the color of the body in the host. But other investigators were not successful in repeating these experiments, and according to Przibram no connection takes place between the nerve strands of the grafted head and the body.

As to echinoderms, these organisms are very unfavorable for transplantation experiments on account of their rigid integument, and very few investigations have been reported. However, Przibram succeeded in homoiotransplantation of the disc in crinoids; also in transplanting this organ into other varieties differing from each other in their color; and even in the starfish H. D. King accomplished, in one exceptional case, a homoiotransplantation, in which however the ectoderm of host and transplant was the only tissue which underwent union.

As far as we can judge from the relatively limited number of experiments in arthropods and echinoderms, these organisms seem to behave in a similar way to lumbricidae as far as manifestation of organismal differentials is concerned, provided we disregard more or less accidental difficulties in transplantation due to peculiarities in the structure of these animals. On the other hand, the difference between the results of homoiotransplantation and heterotransplantation of ovaries in the experiments of Kopée indicates that, after all, the sensitiveness to heterodifferentials may be greater in this class than in lumbricidae, and that the reactions of the host against the strange transplant may be more complex. We have furthermore to consider the possibility that the relatively low degree of regenerative power which these organisms possess may render the manifestation of a reaction against transplants possessing a different organismal differential more difficult than in lower organisms.

In evaluating the relative significance of organismal differentials in the various classes of animals which we have analyzed so far, we must in addition to the complications already mentioned, take account of the fact that our estimates as to reactions against strange differentials are based largely on a gross study of the grafts. A study of the finer cellular reactions, which may have occurred, is lacking, and if undertaken it might have made possible a finer gradation of the organismal differentials. As stated, auto- and homoiotransplantation show no marked differences in results in these various classes of animals, and even heterotransplantation succeeded in a number of cases between more nearly related species. In these respects the different classes so far considered have behaved in a similar manner.

Much more pronounced were the differences in the rigidity of organization and in the possibility of inducing new organ formation or of transforming one organ into another in these types of animals. As regards these reactions, we find a definitely graded series, beginning with the hydroids and ascending by way of planarians to the annelids and then to the arthropods. In the latter, only small parts of the body can undergo far reaching changes; we refer in

latter. In all probability we have therefore in these cases to deal with a result similar to that found in lumbricidae; heterotransplantation may succeed, yet heterodifferentials do exist and exert a certain influence on the fate of the transplants. On the other hand, we must always consider the possibility that whenever negative results were obtained in heterotransplantations, this may have been due not to a primary incompatibility of the organismal differentials, but to secondary factors, as for instance, to differences in the size of the pieces to be united and to similar more or less accidental conditions.

The subsequent experiments of Meisenheimer also make it probable that heterodifferentials may play a role in these transplantations. In Lymantria this investigator succeeded in transplanting ovaries into individuals of the same, as well as of different species, provided the latter were nearly related. We might then conclude that heterodifferentials become manifest only in the case of more distant species, either because in more nearly related species the less marked differences in genetic constitution do not lead to the production of antagonistic mechanisms to the same extent as greater differences, or because the sensitiveness and reactivity to the injurious action of the corresponding heterodifferentials are less pronounced in these lower forms than in higher organisms. However, the experiments of Kopéc, which followed those of Meisenheimer, indicate that heterodifferentials are well developed in moths; he found that, while homoiotransplantation of sex glands succeeds, heterotransplantation does not. Of interest is also his observation that different tissues show different degrees of resistance to the manifestation of heterodifferentials; thus the germ cells are the most sensitive; these die earliest after heterotransplantation, while transplanted connective tissue grows at first and is only secondarily destroyed. Furthermore, he also notes that the destruction of the transplants takes place the more rapidly, the more distant the heterogenous species are from each other. It seems, then, that in these cases we have to deal with a direct injurious action of heterotoxins; but in addition cellular mechanisms participate in these processes, inasmuch as phagocytic cells of the host may destroy isolated heterogenous germ cells.

In insects, according to E. Ries, it is possible to transplant larval fat tissue which has been transformed into a mycetoma, into larvae of different orders. Accordingly, if transplanted from Periplaneta into the peritoneal cavity of Tenebrio, or from Psylla to Tenebrio, the grafted fat tissue remains alive throughout the life of the larva; however, as a result of the strangeness of the transplant, lymphocytes soon begin to collect around it. The transplanted tissue can even survive the metamorphosis of the host into a pupa without being affected by the general changes, and not even by the histolytic processes which occur during this period. In evaluating these observations we must, however, consider the fact that in all the transplantations in insects discussed so far, we have to deal with transplantations not in adult forms, but in caterpillars and pupae.

Some remarkable successes in transplantation have been reported by W. Finkler. According to this author, it is possible in the insect species Hydrophilus to replace the head which has been cut off, by grafting the head of

another individual of the same species; this kind of transplantation apparently succeeded also between different species if they were nearly related (Hydrophilus and Dytiscus), but not between farther distant species. Finkler states that the head thus transplanted determines to a large extent the sexual reflexes and the color of the body in the host. But other investigators were not successful in repeating these experiments, and according to Przibram no connection takes place between the nerve strands of the grafted head and the body.

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particular to the changes in the head appendages which may be induced in certain crustaceans and in which the activity of the nervous system is of importance; here, also, regeneration is on the whole limited to the extremities and other appendages.

In general, the transformation from one part of the body into another, or from one organ into another, seems as a rule not to be a direct one, but this change is apparently accomplished by the return of the part of the body or of the organ involved to a more indifferent state, which subsequently assumes the characteristics of the new part or organ. Furthermore, it appears that with increasing phylogenetic development, cells with relatively great potentialities of further differentiation may take over the newformation of organs.

C. *Transplantation and Individuality in Adult Amphibia*

In our discussion of organismal differentials in amphibia, we shall omit, for the present, transplantations in which host and transplant, or host and partner, are embryonal; these we shall consider later. Furthermore, some transplantations in amphibia were used for the analysis of the factors underlying metamorphosis, and these investigations will also be considered separately. There remain for our present purpose a number of transplantations in adult urodeles and anurans, two groups in which the results differ in certain respects. Of special interest are the transplantations of pigmented to white skin and vice versa, because the behavior of the pigment may serve as an additional indicator of the reaction of the host against the graft.

In the urodele, Triton alpestris, homoiotransplantation of skin succeeded well, as did likewise heterotransplantation of skin from Triton alpestris to Triton cristatus, but in the latter type the healing took place more slowly than in homoiotransplants. The white transplant assumed gradually the dark color of the host skin. On the other hand, Triton salaratus did not, as a rule, tolerate transplants from Triton alpestris but casted them off (Taube). In the salamander, Diemictylus viridescens, Collins and Adolph did not observe a difference between the results of autogenous and homoiogenous transplantation of skin; both remained preserved, but in both, a re-organization of the pigmentation took place.

In anurans, the differences between the results of auto-, homoio- and heterotransplantation of skin were more pronounced. We may here refer also to transplantations in frog tadpoles, where autogenous transplants of white skin to pigmented areas remained preserved, but very slowly, pigmentation could occur in the graft, caused apparently by changes which took place in the transplanted epidermis itself. After homoiotransplantation, the skin healed in more slowly, a relatively rapid invasion of the white skin by the pigmented cells of the host occurred, and lymphocytes accumulated underneath the transplant (Cole). In adult Rana pipiens, according to Hadley, autogenous surface epithelium and glands healed in well, whereas homoiotransplantation of skin succeeded not as readily, the number of unsuccessful grafts being greater and the pigmented cells of the host growing into the graft. Still less favorable were heterotransplantations from Rana pipiens to Rana clamitans.

The healing-in took place with greater difficulty and pigmented grafts that remained attached to the host for more than 15 days became progressively lighter, whereas the unpigmented grafts were invaded by the pigmented host skin; degenerative changes took place in the transplant and large masses of leucocytes collected underneath it. There were then, in anurans, graded differences in the tolerance of the host to autogenous, homoiogenous and heterogenous transplants, and the reactions against strange organismal differentials were, here, more definite than in urodeles. It is of interest that heterotransplantation into the lymph-sac succeeded, and this suggests that it was the action of the tissues rather than that of the bodyfluids which caused the injury of the heterogenous graft.

We may mention in this connection also similar experiments of May in reptiles. In chameleon, May found that autotransplantation of skin succeeded very readily, without any change in the pigmentation of the transplants. On the other hand, homoiotransplants were absorbed after they had healed in, the total absorption taking place between the 61st and 90th day. Usually no change in pigmentation occurred here, except in one case, where the transplant became lighter. Conditions were then, in this case, similar to those seen in birds and mammals, except that the reactions occurred more slowly; whether or not lymphocytes participated in these experiments in reptiles is not stated.

The results of transplantations of other organs are in essential agreement with those we have mentioned. Thus, in the salamander, Diemictylus viridescens, Stockard found that homoiotransplanted pieces of the ovary can be maintained alive in a satisfactory condition in the testicle, but not in other organs. However, in other urodeles, Harms showed that not only homoiotransplantation, but even heterotransplantation, of the ovaries may succeed within a certain range of relationship, and that in general there seems to be a parallelism in the transplantability of tissues and the possibility of hybridization between certain species. In this connection, Harms made the interesting observation that in urodeles blood vessels of heterogenous origin supply the circulation in these transplants, and that the peritoneal cells lining the grafted ovaries may swell, send out processes in the direction towards the lining peritoneal cells of the host, and that both may then meet and coalesce. In adult urodeles, therefore, heterogenous cells may enter into close contact with each other, or may coalesce apparently without the development of any antagonistic reaction. Meyns noted a similar coalescence of cells also in anurans, but it occurred in homoiogenous transplantations. In case a destruction of the heterotransplanted ovary did take place, this may not have been altogether a direct effect of heterotoxins, inasmuch as connective tissue and phagocytic cells of the host took part in the disintegration and elimination of those transplanted ova which had escaped the direct toxic action of the bodyfluids of the host. It seems, therefore, that ova which, under the influence of the strange organismal differential were changed in their metabolism, secondarily were exposed to the injurious action of host cells.

In mammals we observed in certain cases that reciprocal transplantations

did not behave in the same way; similarly, in urodeles some differences have been noted; thus, transplantations of the ovaries from Triton alpestris to Amblystoma tigrinum succeeded, while in the reciprocal transplantation the graft exerted a toxic effect on the host. However, it is possible that in this instance we have to deal not with the direct effect of the organismal differentials, but with specific toxic substances, the production of which is limited to certain organs.

Also, heterotransplantations of testicle were successful in some species of Triton (Koppanyi). But if certain organs are grafted with greater difficulty, then it is possible to make homoio- and auto-, but not heterotransplantations. Thus, according to Kurz, limbs can be homoiotransplanted in adult Triton and even regeneration may take place in autogenous as well as in homoiogenous grafts of this kind; but heterotransplantation does not succeed. Similar observations have been made by Mathey in the case of transplantation of the eyes in Salamander larvae and in adult Tritons. Under these conditions even autotransplantation succeeds only in a small minority of cases, and still greater difficulties are encountered in the case of homoiotransplantation. In Triton, the presence of a functioning spleen, or of substances given off by this organ, may be an unfavorable factor in the transplantation of this tissue. Ehrenpreis accomplished, therefore, a homoiotransplantation of spleen only in urodeles in which the spleen had previously been extirpated; but even in this case autotransplantation seems to have been preferable to homoiotransplantation (Jolly and Lieure).

If we compare the range in which transplantations are possible in anurans and in urodeles, we find a greater restriction in the former. Welti succeeded in homoiotransplantation of the ovaries in Bufo vulgaris, while transplantations into different races failed. The successfully transplanted ovaries gave off hormones which modified certain secondary sex characters in the host. Meyns observed that the testicle is readily homoiotransplanted in immature frogs, but can be heterotransplanted only in exceptional cases. In adult frogs even homoiotransplantation does not produce as favorable results as autotransplantation, an observation which indicates that also homoiodifferentials may exert here an injurious effect on tissues. Furthermore, this investigator noted, in accordance with the findings in the case of mammalian organs, that different constituents of an organ may differ in the degree of sensitiveness which they manifest; the efferent ducts of the testicle were less sensitive to the injurious effects of transplantation than the generative cells proper. As in Tritons, so also in anurans a specific hormone may inhibit the successful transplantation of an organ with an internal secretion. Thus transplantation of the testicle was possible neither in normal males nor in normal females, but only in castrated animals. Which phase in the process of transplantation is here affected by the internal secretion, whether it is the healing-in of the graft or its subsequent preservation, is not clear from the data on hand; nor do we know whether the destruction of the transplant takes place directly under the influence of the injurious bodyfluids of the host, or through the mediation of the host lymphocytes and connective tissue cells.

An internal secretion is also active after homoiotransplantation of thumb-pads of the frog; however, in this case the testicular hormone affects only the further growth processes in the organ after it has healed in; and according to Harms, it accomplishes this effect largely by means other than variations in the circulatory condition and blood supply received by the transplant.

Rhoda Erdmann noted that in the adult Rana autotransplantation of skin succeeded well; but homoiotransplantation succeeded only for periods of from two to four months, and skin glands did not develop in the latter kind of transplants. But even after heterotransplantation from Rana temporaria to Rana arvalis the graft could remain attached to the host for as long as eighty days, when it was cast off. Similar to the observations of Harms as to toxic effects exerted by ovaries transplanted into Triton, and to those of Dürken in the transplantation of parts of neurulae into larvae of Rana, Erdmann noted after transplantation of skin of Hyla to Rana, the occurrence of hemorrhages and other toxic symptoms in the host. These results agree also with those of Schultz, who found that after transplantation of skin from Bufo viridis to Bufo vulgaris, the host died, while the reciprocal transplantations were successful. It is evident that specific toxins given off by such transplants complicate the results of grafting in these amphibia, and that we have not merely to deal with the effects of organismal differentials. As stated above, similar effects have also been observed after transplantation in certain urodeles.

We may then conclude that heterotransplantations succeed with greater difficulty in urodeles than in lower classes of animals, and with still greater difficulty in anurans. In the latter class some differences have been recorded even between the results of homoio- and autotransplantation, and we may then conclude that a furthergoing stage in specialization of these differentials has been reached in amphibia, and that within the amphibia there is seen a graded advance in the refinement of the organismal differentials in the transition from urodeles to anurans. However, as we pointed out previously, the lack of an unfavorable reaction of a host against a certain kind of transplant does not exclude the presence of the finer grades of organismal differentials. Thus we cannot exclude the possibility that in urodeles, as well, individuality differentials may exist; indeed, certain observations indicate that here, also, autotransplantation may succeed better than homoiotransplantation.

The interpretation of the results of transplantations in the more primitive organisms which we have discussed so far, is based largely on the gross examination of the transplants; however, in the case of grafting in the urodele Triturus, Anderson and Horowitz have carried out microscopical examinations, in which they compared the reactions in auto-, homoio-, and heterotransplantations of skin (Anderson), and muscle tissue (Horowitz). Horowitz has described a reaction of the fibroblasts and lymphocytes of the host, which invaded the transplant the more actively, the stranger the organismal differentials were between host and transplant. In the case of heterotransplants, also polymorphonuclear leucocytes participated in this reaction. These various cells succeeded in destroying tissues possessing organismal differ-

entials incompatible with those of the host. In principle, conditions are therefore similar to those found by us in the case of mammalian tissues; a difference exists only in that the reactions in urodeles were slower than in rodents, and also in that lymphocytes and fibroblasts later destroyed parts of the autotransplants, while other parts remained preserved; these reactions against autogenous tissues were presumably due to necrobiotic changes which occurred in certain areas of the grafts. In both urodeles and mammals the vascularization of autotransplants was better than that of homoio- and heterotransplants.

Also, Hitchcock finds that frog skin autotransplanted into the lymph sac of frogs remains preserved much longer than heterotransplanted skin. Ultimately, however, it is destroyed through the ingrowth of fibroblasts; but this result is due merely to accidental factors and not to factors inherent in amphibian tissues, as is evidenced by the fact that skin autotransplanted into a defect of skin remains preserved indefinitely. Similar results may be obtained in mammals and birds, for example, when skin is injured after autotransplantation into the subcutaneous tissue. Following heterotransplantation of frog skin, the transplant is destroyed much more rapidly than after autotransplantation and the destruction takes place the more rapidly the farther distant phylogenetically the species of host and transplant; frog skin dies very quickly after transplantation into Triturus and Triturus skin becomes necrotic within a very short time after transplantation into the frog. After transplantation into urodele species, it is the heterotoxin of the bodyfluids which kills the transplants, while after heterotransplantation into Rana, the injury is due, above all, to the action of leucocytes. In the tissue surrounding the graft, lymphocytes accumulate. Conditions here are therefore, in principle, similar to those after heterotransplantation of mammalian and avian tissues; only in the latter the injurious action of the bodyfluids is evident in every instance, while, according to Hitchcock, this effect is not noticeable after transplantation of frog skin into more nearly related species. Presumably we have, in the case of frog skin, to deal merely with quantitative differences in the effects of toxins and of cellular reactions, such as were observed also in the case of mammalian tissue, where we noted that heterotransplantation of cartilage produced cellular reactions which were much more prominent than those following heterotransplantation of such very sensitive tissues as thyroid and kidney, which are destroyed by strange bodyfluids within a very short time. We have found also other instances of quantitative differences between the respective importance of toxic serum and cellular reactions in different species of mammals. It has been assumed in the case of tumor transplantation that necrosis primarily attacks the center of the pieces and not the peripheral parts, which indicates that an injurious action of the bodyfluids on the transplant is lacking. Hitchcock uses the same argument in order to prove the absence of an injurious action of the bodyfluids after transplantation of skin of Rana into strange species of Rana. Nevertheless, homoiotoxic action does exist in the case of tumors as well as of normal mammalian tissues; and we may draw the same conclusion in regard to heterotoxic action in heterotrans-

plantation among anuran species. The center of the graft degenerates first because it is injured by the lack of foodstuffs; it succumbs therefore before the peripheral parts do, which resist the injurious action of the bodyfluids better, since they are near the source of oxygen and the foodstuffs. Careful microscopic studies of grafts in amphibians tend to prove, therefore, that here, in principle, already a fargoing specialization of the organismal differentials has taken place and that this specialization manifests itself in a similar manner in amphibia and in mammals.

In progressing from the urodeles to the anurans there is thus noted an advance in the specificity of the reaction on the part of the host against a strange organismal differential. Furthermore, parallel to this progression in specificity a reverse change takes place in the regenerative and integrative power of these classes of animals. While this is very much more restricted in urodeles than in the primitive invertebrates, still a certain degree of the power of regeneration has been retained by them, as indicated by their ability to regenerate extremities. In the anurans, on the other hand, only the rudiments of this integrative power are left, consisting merely in the ability of a number of individual tissues to undergo, to a moderate degree, proliferative processes, which may lead to the filling-out of certain defects and to wound healing in the skin. In accordance with our previous conclusions we find, therefore, also in this instance a parallelism between the degree of plasticity in the organ-forming potencies of organisms and the development of the finer organismal differentials. The greater the transformability of organs and the greater the restitutive and integrative power of organisms, the more undifferentiated appear to be the organismal differentials and the less specific are their effects.

From this brief survey of the behavior of organismal differentials during phylogenetic development we may conclude that already in the most primitive organisms certain reactions against tissues from strange species are present, and that these reactions become more refined and specific with the advance to groups of animals whose structure is more complex. But we observe also that with progressing evolution the differentiation of organs and tissues, their decreasing plasticity and increasing fixity are much more clearly graded than is the refinement of the organismal differentials. No definite advance was observable in the individualization of organismal differentials within the most primitive classes of animals; they all seemed to behave in a similar manner. However, this lack of steady progression is perhaps only apparent and not real. It may be at least due partly to the method used for the demonstration of the organismal differentials, namely, observation of the reactions which take place in an animal against a strange transplant, or between two partners differing in their organismal differentials; now, in the lowest organisms the tendency to integration is very great and there is the danger that the integrative reactions which follow various kinds of disturbances may be interpreted as reactions against strange organismal differentials. Furthermore, we may recall the great complexity of the factors which enter into the reactions against strange organismal differentials, and the fact that the intensity of these reac-

tions is determined not only by the presence of the finely differentiated substances, which represent the organismal differentials in tissues and organs, but also by the rate at which such substances are produced, given off by the cells and allowed to diffuse into the strange organism; finally, the results of the reactions vary with the sensitiveness of the tissues involved. As far as the injurious effects of incompatible bodyfluids are concerned, our ability to discern these depends mainly on the sensitiveness of the tissues on which they act; on the other hand, the strength of the cellular reactions against tissues with strange organismal differentials is, to a certain extent, susceptible to measurement, even if the tissues are more resistant.

We see, thus, that we have to deal with a considerable number of factors if we wish to grade the degree of compatibility of the organismal differentials of different organisms. With regard to the higher organisms, only a beginning has been made in the approximate quantitative grading of these incompatibilities; in the case of the more primitive organisms the observations which bear on this question are more or less casual and are based largely on gross aspects of the changes following transplantation. The introduction of microscopical methods for the study of cellular reactions against strange differentials, methods similar to those used in the case of mammalian tissues, has only recently been undertaken in the study of transplantations in urodeles. The results already indicate that reactions against strange tissues, endowed with strange individuality differentials, are present in classes of animals where the methods formerly used were not adequate for their demonstration. In addition, there are the immunological studies of the relationship of the bodyfluids of various organisms; these are based on the ability of bodyfluids to serve as antigens and to call forth the production of antibodies, which latter, in their interactions with antigens, indicate the relationship between various species of animals. These investigations will be discussed in a subsequent chapter.

We may then conclude that in the most primitive classes of animals the substances which represent the organismal differentials or at least the reactions against these differentials are as yet less finely differentiated than in higher organisms, and that in general there is a correspondence between the lack of a finer differentiation of organismal differentials and the lack in the finer differentiation of organs and tissues, and an inverse correspondence between the degree of development of organismal differentials and the degree of plasticity of these organs.

As stated above, the graded progression towards an increase in the specificity and fixity of organs and tissues in the course of evolution are clearly discernible. With advancing phylogenetic development the various parts of the body differentiate more and more into a variety of organs, tissues and cells, interacting with each other according to patterns which are specific and rigid. Hand in hand with this change from the relatively simple structures of such primitive organisms as the hydrozoa, to the greatly differentiated types of organs, tissues and cells of the anuran amphibia, there takes place a change also in the kind of substances which regulate the interaction of the different parts of the individual. In hydrozoa we must assume that substances repre-

senting various kinds of inductors exist and are transferred from one part of the body to another; these, acting on a specific substratum of a very plastic nature, are presumably responsible for the production and growth of the organ systems which are characteristic of the different regions of the body. In arthropods such transformations are limited to certain appendages, and also in urodele amphibia integrative processes are possible only within a very limited range; but in anuran amphibia they are lacking altogether. There develop in insects and in the higher organisms, in accordance with their more finely differentiated organs and tissues, hormones and neuro-hormones which affect certain organs and cells in a very specific manner. Such hormones may also affect the life and growth of transplanted organs with which they have specific relations. Perhaps a corresponding increasing differentiation exists also in the case of various other substances, such as vitamins and enzymes, which regulate maintenance, growth and metabolism of organs and tissues; but we have as yet no definite knowledge as to the phylogenetic development of the latter types of substances.

Chapter 3

Transplantation and Individuality of Embryonal Tissues

We have studied the phylogenetic development of organismal differentials and their manifestations in animals, using transplantation of adult tissues as indicator. In this and the following chapter we shall study the ontogenetic development of the organismal differentials and for this purpose we shall make use of the data supplied by the transplantation of embryonal tissues. In these experiments either parts of developing organisms were joined together, each of which was capable of independent life, or relatively small, not independently viable pieces of embryonal tissues or organs were transplanted into embryonal or adult organisms. The union of independently viable parts bears some resemblance to parabiosis especially if the size of the surface, by means of which the partners are joined together, is relatively small in comparison with the diameters of the grafts.

I. *Transplantation in Amphibia.* It was in amphibia that the possibility of uniting parts of two different embryos into one organism was discovered in 1897 by Born, whose work thus introduced a problem which subsequently suggested many similar investigations in amphibia as well as in other classes of animals.

Born used in his experiments larvae of anuran amphibia. In these as in other transplantations, besides the organismal differentials, other factors, some of which were non-specific, helped to determine the results, and it is important, as far as feasible, to separate these factors. Thus, the rapidity of growth of larvae of Rana esculenta is greater than that of larvae of Rana fusca or arvalis, and in the union of parts of the former with parts of one of the two latter larvae, components of Rana esculenta tend to dominate over the other components and cause their atrophy. It is necessary to distinguish such secondary effects from the direct manifestations of organismal differentials, although the growth rate is, of course, as well as all other characteristics of tissues and organs, at least in part, determined also by the genetic constitution of the organism.

As a direct effect of the organismal differentials, we may consider the readiness with which autotransplantation of embryonal constituents succeeds: two parts can be readily united into a single organism, in which the corresponding organs form so perfect a connection that, subsequently, the place of junction can, as a rule, no longer be recognized; however, difficulty may be experienced in the joining together of the components of the chorda dorsalis, especially in older larvae. As in adult annelids, so also in amphibian embryos analogous organs of the partners usually find each other and unite.

The results of homoiotransplantation are about the same as those of auto-

transplantation. Here, too, all kinds of combinations succeed, including the union of smaller parts, which as such would not be capable of independent life, with larger parts of larvae; in these experiments abnormalities, such as organisms possessing two heads, may develop under certain conditions. Also, in homoiotransplantation analogous organs tend to find and join each other and usually it is impossible to recognize later the original line of demarcation. No incompatibilities due to differences in individuality differentials develop, and such combinations of organisms may even pass through metamorphosis. If difficulties do arise, they are of a non-specific nature.

Similar were the results in heterotransplantation if the partners belonged to nearly related species. In this case also the analogous organs of the two partners had the tendency to unite and thus the two partners developed into one homogeneous organism, in which no scar could be recognized at the point of union; but when non-analogous organs of embryonal partners happened to join, a scar did form, or else the organs separated after some time. As we have noted previously, in the joining-together of pieces of adult lumbricidae there developed at first a scar, which only secondarily was replaced by the specific tissues. While the rates of growth in the two partners could be independent of each other, the rates of differentiation were about the same, substances circulating in both partners determining presumably the latter effect. As we shall see later, Uhlenhuth, in transplanting eyes in salamander larvae, found a similar correspondence in the rate of differentiation and in the time of metamorphosis of host and graft. There resulted, thus, not only a harmonious union of the two embryos belonging to different species, but in certain cases even the blood vessels of one partner could grow into the other partner apparently without causing any incompatibility.

We find, then, that heterodifferentials do not need to prevent the direct union of the specific tissues in analogous organs without interference by connective tissue; this was true also of parts of the nervous system, even in cases in which the diameters of the components differed in the partners. On the other hand, if non-analogous organs happened to meet, as stated, the union took place by means of connective tissue, except in the case of ectodermal and entodermal epithelia. These observations suggest that under these conditions tissue differentials functioning as contact substances regulate the interaction of tissues from analogous organs at the point of junction, although the species differentials of the corresponding tissues differ in the two partners.

While after union of embryos from different species of Rana, the results were similar to those obtained in homoiotransplantation, such combinations were not able to maintain themselves for longer than two to three weeks when species as distant as Rana esculenta and Bombinator igneus were united. Although for some time in the beginning the partners could develop normally and the double organisms begin to feed themselves, after awhile they became sickly and progress ceased. Therefore, in the case of transplantation of more distant species, heterotoxins apparently led to various abnormal conditions in the animals. In Born's experiments circulatory disturbances became manifest after about fourteen to sixteen days; there was either edema or no

circulation at all and death followed. Also in the subsequent experiments of Braus, who showed that under favorable conditions the life of such combinations could be prolonged for as long as five weeks, ultimately serious incompatibilities developed. However, union between members of different orders (urodeles and anurans) did not succeed for longer than one or two days.

When thus heterotransplantations between nearly related species succeed well, this does not necessarily mean that heterodifferentials do not exist in their tissues, but merely that the intensity of the reaction against the strange organismal differentials does not preclude a successful transplantation. However, if the conditions under which such heterotransplantations take place are less favorable, then the existence of incompatibilities between the heterogenous organisms may become manifest. Hence, while the parabiosis-like union between different species of Rana could be readily accomplished, exchange of pieces of skin between the larvae of different species of Rana did not succeed; within a few days the grafts became smaller and then disappeared. As for the raising of such combinations of embryos to a stage further than metamorphosis, Born succeeded only in the case of homoiogenous grafting of embryos of Rana esculenta. He did not succeed in reaching this stage with heterogenous combinations, although in other respects, as noted above, heterotransplantations between nearly related species behaved about like homoiotransplantations.

The experiments of Born were continued by Harrison, who in one instance kept alive a heterogenous combination (Rana virescens and Rana palustris) through the period of metamorphosis and was able to observe that each of the two constituents in this combination retained its characteristic species features. But the size of a whole animal of this kind was much smaller than that of a normal frog. In general, such heterogenous combinations, although able to eat and shift for themselves, became weak in the course of time, they decreased considerably in size and finally died; at most, they could be kept alive for three or four months, while in Born's experiments similar heterotransplantations succeeded only for a period of three weeks. But even in Harrison's experiments atrophy and degeneration in the large majority of cases set in after a few weeks. This investigator also observed that if a tail had been grafted to an individual of a different species, there was noticeable an early interference not only with the growth, but also with the life of the grafted tail, parts of which, however, could remain viable for a longer time.

Harrison furthermore noted that in some instances reciprocal transplantations behaved in an unlike manner, an effect which has been found also in other kinds of transplantations and to which we have previously referred. Of interest also is his observation that the lateral organs of one partner could grow into the other, although the partners belonged to different species, as happened when the tail portion of Rana palustris was joined to the anterior part of Rana sylvatica; in this case the lateral line organs extended from Rana palustris into Rana sylvatica. Evidently there was here no very marked incompatibility between parts of organs possessing, each one, its own species differential; if antagonistic reactions did occur under these conditions, they

were presumably of a subtle nature and proceeded more slowly. Even individuals belonging to different genera and families could be temporarily joined together.

On the whole, these experiments bear certain similarities to mammalian parabiosis; apparently heterotoxins are active in both. Inasmuch as in these transplantations of parts of embryos we have to deal, not with the peculiarities of some of their constituent tissues or organs, but with conditions common to all the tissues, which are affected the more unfavorably the greater the distance in relationship between the two partners, we are justified in attributing the incompatibilities which may develop between them to differences in their organismal differentials. These embryonal organisms show a sensitiveness to heterogenous differentials similar to that noted in certain invertebrates, as for instance, the lumbricidae; in both cases incompatibilities arise if the species of the partners are far removed from each other phylogenetically. The mutual tolerance of heterogenous constituents seems to be greater in the embryonal than in the adult anuran amphibia, which latter, as we have seen, are on the whole very sensitive to the effects of heterotransplantation. We have seen, in a preceding chapter, that in adult amphibia restitution processes are restricted to the appendages of urodeles. On the other hand, in amphibian larvae of Rana, Harrison has shown that it is possible to obtain furthergoing integrations. When pieces of tail were grafted so that their aboral poles were in contact with the oral poles of the host and the oral surfaces of the grafts were cut off, the influence of the larger piece induced processes of adaptation in the grafts, which made them part of the host. In this respect a larva of an anuran amphibian resembles, therefore, a hydrozoon or a planarian; but in other respects the integrative ability of these larvae is much less pronounced than that of the more primitive adult invertebrate organisms. As a rule, in amphibian larvae abnormal combinations of several pieces do not undergo those various regulative processes leading to the reestablishment of normal individuals, which take place so readily in primitive adult animals; the larvae of amphibia correspond in this respect rather to adult lumbricidae.

II. *Transplantation of Embryos and Eggs in Invertebrates.* The experiments of Born in amphibia were soon afterwards extended to invertebrates. It is especially the eggs and embryos of echinoderms, of Ascaris and Chaetopterus, which were used in these investigations, in which Driesch, Morgan, zur Strassen, Jacques Loeb, de Haan, Goldfarb, and others participated. Although these experiments were not undertaken primarily for the sake of the study of organismal differentials, still some valuable data in this regard were obtained.

Two cells or cell complexes may be joined together in two ways: (a) Through agglutination, a process which will be more fully discussed in a later chapter, dealing with tissue formation and organismal differentials; (b) through coalescence of agglutinated cells, due to solution processes which take place in the ectoplasmic cell-layer, especially of eggs or their very early cleavage stages. If the union consists merely in an agglutination process, several further possibilities exist. Either the two organisms remain distinct and

develop as separate embryos, or they become secondarily integrated into a single organism through the action of regulating mechanisms,—presumably similar to those which are effective as contact and distance substances,—on cells derived from the same embryo, and then an orderly development may follow. In addition there may be observed certain intermediate conditions in which the greater parts of the two embryonal structures remain distinct, but some organs unite and become common to both organisms. Furthermore, under certain conditions the joined organisms may separate again secondarily, the result of a process which may be designated as disagglutination. Structures representing various stages of embryonal development can thus be united, unfertilized or fertilized eggs as well as early cleavage stages up to blastulae, and perhaps even still farther advanced embryos.

If two embryos have in this way been joined together by means of agglutination into a single organism, giant individuals may develop, in which the number of cells composing the embryo is approximately doubled, but in which the size of the cells remains unchanged. However, in other cases in which, at a very early stage, coalescence takes place between the two partners, an organism with the same number but with double the size of cells results. Under certain conditions it may happen that one of the two organisms becomes atrophic and then the remaining parts of it may be dominated by the larger partner. Such a dominance of a larger over a smaller partner has been noticed repeatedly in cases of transplantation in lower invertebrates, as well as in parabiosis in mammals. Whether the two embryos will form one single organism or separate into two organisms depends upon several factors: (1) The degree of development of the embryos and the rigidity of their tissue and organ differentials at the time of union; in general, the further the embryonal development has progressed, the more the original plasticity of tissues and organs has been lost, the less will be the chance that one single individual will result from the union. (2) The orientation of the two surfaces which unite the two partners; if this orientation is favorable then the union, whether by means of agglutination or of coalescence, can be more readily accomplished and a secondary separation becomes more improbable. This conclusion agrees with observations in lower adult invertebrates, where the covering of wound surfaces in the right orientation prevents regeneration at the cut ends, but where the joining together of two unsuitable poles may lead to budding or regenerative outgrowth and subsequent separation of the component parts. It agrees in general also with the changes which take place at the point of union between tissues in higher animals, where certain contact differentials determine whether a stable or an unstable equilibrium will be reached. (3) The result also depends upon the organismal differentials of the two partners. Syngenesious and homoiogenous combinations apparently succeed. However, in many cases it is impossible from the reports of the investigators to determine whether, in a certain experiment, a syngenesio- or a homoiotransplantation was carried out, and we can therefore not be sure whether any difference existed between the results of these two types of transplantation; but some investigators, and in particular Bierens de Haan,

have given consideration to the influence of organismal differentials in their transplantations.

More definite is the difference in effects which is seen between homoio- and heterotransplantation. After the latter, there may take place neither a primary nor a secondary unification of the two organisms. The incompatibility may manifest itself at the surfaces where the organisms are joined together and thus a separation, due to disagglutination, may occur after apparently a primary union of the organisms had taken place. If we unite two distant species, either this latter process occurs or there may be from the beginning a lack of union. If, however, we combine more nearly related species, the two organisms may remain united for a longer period of time, but secondarily also here abnormalities occur, such as a slowing-up of the developmental processes, until they cease in the end altogether. In still other cases more localized abnormalities in development take place, affecting either one or both of the partners; or on the other hand, disintegration or atrophy of tissues has been observed and at last one partner may be destroyed or incorporated into the dominating one. In these instances we have, therefore, presumably to deal with heterotoxins injuring especially the weaker organism. However, the difficulties experienced in transplantations between different species may depend not entirely on the incompatibilities between the organismal differentials as such, but also on secondary factors of a less specific character, such as differences in the size and rate of development of the two partners; factors of this kind may determine the readiness with which two relatively nearly related species can be joined together.

Of great interest is the observation that in heterotransplantation, if a part of one of the two component organisms disintegrates, the remaining part of this organism may be changed in its development under the influence of certain organs of the other partner, which has now become the dominating factor. Thus, a line of ciliated cells may form in the injured component of the combination, when similar developmental processes take place in the dominating component. In such a case we have apparently to deal with an organizer action similar to those actions which are potent during the normal development of embryos, or which may be produced experimentally through implantation of certain specific parts of another embryo, which function as organizers. Evidently the presence of heterodifferentials does not necessarily prevent organizer action.

We see, then, that in the case of invertebrate embryos a distinct sensitiveness to heterogenous organismal differentials exists, while a like sensitiveness to homoiogenous organismal differentials is apparently lacking, and in this respect eggs and embryos behave in a similar way to parts of adult invertebrates when they are joined together. However, as stated above, it may be that what has been interpreted in these experiments as homoiotransplantations, really represented syngenesiotransplantations, since this distinction was not always made by the investigator.

We shall cite a few experiments which will illustrate some of the general conclusions at which we have arrived and which will bring out some addi-

tional points of interest. In certain instances ova were joined together and agglutinated with each other, as in the experiments of de Haan and zur Strassen; in others, early embryonal stages were combined (Driesch, Goldfarb).

Driesch united two blastulae of Echinidae and observed that they were able to form one organism with twice the number of cells, but the individual cells did not coalesce. A single organism developed presumably if contact mechanisms or contact substances acting as regulators were favorable to such a union; otherwise, separate organisms resulted. Apparently the stage at which the joining together of the component parts took place and the size of the surfaces which agglutinated (Goldfarb), and probably also the degree of specificity of tissue and organ differentials, determined the outcome of the operation. The further the differentiation had progressed, the more pronounced was the tendency on the part of the partners to separate again and to give origin to two distinct organisms. If one organism had been produced, the inner organs appeared to be double or they had united into single organs. In some cases two larvae formed, which had certain organs, such as gut, skeleton or body cavity, in common, while in other cases these organs remained separate. When one partner dominated, as so often occurs in mammalian parabiosis, distintegration of the skeleton could take place in the weaker partner, and as a result of such degenerative processes a single larva developed, in which the gut of the dominant larva supplied the remnants of the other partner with food; furthermore, it was observed that even mesenchyme cells could move from one larva into the other. Agglutination was, in these experiments, preliminary to coalescence and we may assume that it took place readily if the consistency of the cells was suitable for this process. According to Herbst and Driesch, lack of Ca and a certain degree of alkalinity or low temperature in the surrounding medium caused stickiness and favored agglutination of the cell surfaces. In Driesch's experiments it is not indicated whether he had to deal with syngenesious or homoiogenous relations between the partners; but in Goldfarb's experiments, both syngenesious and homoiogenous unions succeeded. In the investigations of Morgan in Echinidae, parts of brothers were successfully joined together in syngenesious transplantations. He observed that processes of degeneration or atrophy in one of the partners could precede the transformation of the combination into a single larva, but there were also found all transitions between a single homoiogenous organism and double organisms. Goldfarb, as well as Bierens de Haan, showed in Echinidae that as many as forty eggs could be made to agglutinate with one another, but that a combination of more than two eggs rarely developed beyond an early embryonal stage. Thus an incompatibility became noticeable, comparable to that observed in transplantation of primitive adult invertebrate organisms, where likewise the difficulty in the integration of the parts into one whole seemed to increase the more the greater the number of pieces which were joined together.

Of general interest are also the experiments of zur Strassen, who showed that in Ascaris two unfertilized eggs could coalesce, and that there was present

under these conditions a double set of chromosomes as well as twice the amount of egg substances. Such eggs could then be fertilized and could develop into one individual. In case two already fertilized eggs were united, the two female and also the two male nuclei, respectively, united with each other, and either twins or single embryos resulted from such combinations. If, instead of eggs, early embryonal stages were combined, they tended to agglutinate rather than to undergo coalescence, the protoplasm of the individual cells remaining separate. However, the direction of the axes of the two organisms was found to be of significance; if there was correspondence of direction, one complete organism developed, otherwise two separate individuals. In general, the two factors which above all others seem to be of importance in such combinations and which determine whether one or two organisms shall be formed from two young embryos are: (1) The developmental stages of the embryonal structures, and (2) the direction of the axes of the organisms.

As to the effects of the organismal differentials, Bierens de Haan found that different combinations succeeded unequally well, it being easier to unite certain species than others. Heterogenous fusions succeeded only rarely, and if they did succeed, the resulting fusion was less complete. In this case there was a chance for a secondary separation of the partners, similar to the separation which had been observed if two more distant species were joined together in primitive invertebrate adult organisms and in amphibian embryos. Separation could still occur as late as after eighteen hours; but if in other cases these heterogenous combinations developed, the development was not quite typical and it was slower than normal. Unified, single giant plutei never resulted from such heterogenous unions. Some combinations of this kind, however, succeeded better than others and in these successful experiments the intestines were seen to grow from one into the other partner. But even in relatively successful heterogenous transplantations, such as those between Parechinus and Paracentrotus, the organ formation was rudimentary and development soon ceased; perhaps substances which were produced as the result of incompatibilities between the two organisms acted as poisons. In a combination between Parechinus and Paracentrotus the dominant Parechinus could call forth the development of a line of cilia at the expense of the rudimentary Paracentrotus, and in this case parts of the skeleton seemed to act as organizers. In general it was found that heterogenous combinations never led to the formation of really uniform organisms, but that at best merely sectorial chimaerae were produced. Under the most favorable conditions each component of these combinations could develop to the stage of a normal pluteus, otherwise regulative processes occurred which led to separation; in other cases, both heterogenous partners became sickly. As far as unfertilized ova are concerned, it was observed that while a homoiogenous union could be accomplished, heterogenous unions did not succeed. From the latter, unified single giant plutei never formed; in some instances cytolysis, in others a temporary agglutination occurred, but a real coalescence did not take place. However, even twin larvae which developed from a homoiogenous union often showed some defects. We

must therefore consider the possibility that specific toxic substances distinct from the organismal differentials were responsible for some of the results following heterogenous, and even homoiogenous combinations.

When we review this entire series we come to the conclusion that there is no very definite gradation noticeable in the joining together of different individuals, if we ascend from the ontogenetically lower to the more adult forms. However, there develops in every case an incompatibility between farther distant heterogenous parts of an artificial combination, and it is often noticeable also between more nearly related heterogenous partners; on the other hand, there is usually no definite incompatibility between homoiogenous partners, although even here some abnormalities may be found.

However, there are several difficulties in interpreting these findings. In the first place, as stated above, these investigations were not carried out with a view of analyzing the organismal differentials and therefore the experimental data which would make possible such an analysis are very incomplete. Systematic comparisons between auto-, syngenesio- and homoiotransplantations were in no case made. Secondly, it is possible that in some instances factors of secondary importance came into play, such as the more or less accidental differences in the size of the surfaces which were to be joined together. Thirdly, there are some indications that organ differentiations and the interactions of organs that adjoined each other played a definite role in determining compatibility; this is suggested by the importance of the orientation of the surfaces of contact. In addition, there may have been active, specific toxic actions, which were referable not to the whole organism as such and to its organismal differentials, but to specific metabolic processes of certain organs, and which were comparable to the toxins produced in the glands of some amphibia and reptiles.

Notwithstanding these difficulties of interpretation, there is very little doubt that essentially the results of the joining together of two ontogenetically primitive organisms depend upon the compatibility of their protoplasms, and in particular, of their ectoplasmic layers, which presumably form around wound surfaces of cells. But, while a coalescence takes place only between homoiogenous individuals, or possibly between individuals belonging to very nearly related species, the primary agglutination process seems to be less specific, although specificity is not lost entirely. Furthermore, we find here, opposed to the tendency to coalesce and to form one unified organism, a tendency towards regeneration and the development of two distinct organisms similar to that which we observed in transplantations among phylogenetically primitive organisms. This applies especially to heterotransplantations. The more suited to each other the character of both the protoplasms and the surfaces of contact, the less this regenerative tendency will assert itself. Instead, integrative mechanisms, which tend to make one single organism out of the two, dominate. While it is impossible to form a definite concept as to the relative importance of physical and chemical factors which may assert themselves at the place of union, still it is evident that the degree of relationship between two embryonal organisms is one of the factors

which helps to determine the compatibility of the partners in experiments in which different species are joined together.

There is reason for assuming that regulative substances of a similar character to those present in the morphogenic interaction between parts of a natural individual, regulate also the interaction between coalesced and secondarily unified organisms. This tendency to form more or less normal individuals out of abnormal combinations may lead to the production of a single organism from two partners, or to the later separation of the two joined-together parts, each of which then gives origin to a single individual, or to the domination of one partner over the other, which latter undergoes various degrees of degeneration. These observations apply both to transplantations in phylogenetically primitive classes of animals and to fusions of early ontogenetic stages. However, notwithstanding these similarities, one has the impression that the regulative and integrative mechanisms, which are so pronounced in the case of the phylogenetically most primitive adult organisms, are perhaps not effective to quite the same degree in embryonal forms of phylogenetically more advanced organisms, although here, also, various fargoing regulations may take place. The typical organizer actions which may be observed in transplantation of very primitive adult organisms, and which are so important during embryonal development, are only very rarely evident under the conditions prevailing in these combinations between ontogenetically primitive organisms.

In experiments with eggs and young embryos we have not to deal with the same organismal differentials which are active in adult organisms. There are indications that neither the specific organismal differentials which characterize the adult individual, nor the mechanisms which react against strange differentials are as yet fully developed; still, protoplasmic specificities which distinguish different species evidently exist even in such ontogenetically early forms. Some of these specificities presumably represent stages in the development of the organismal differentials and their means of manifestation, and all intermediate gradations between the precursors present in the fertilized egg and the fully formed substances and mechanisms in the adult form may be found. Furthermore, as in phylogeny, so also in ontogeny there is noticeable an inverse parallelism between the degree of plasticity of organs and of the integrative potentialities active in an organism, on the one hand, and the the degree of development of the organismal differentials, on the other hand.

Chapter 4

The Significance of Organismal Differentials in the Transplantation of Pieces of Embryonal Tissue into Embryos and into Adult Organisms

IN THE PRECEDING chapter we have discussed transplantation of parts of organisms, each of which had the ability to live and develop independently, in invertebrate and amphibian embryos. We shall now consider experiments in which smaller pieces of tissue, which under ordinary conditions are not able to live separately or to develop, were grafted into embryos or into adult individuals. Transplantations of this kind in amphibia have been used, especially by Spemann and his associates, in the study of the effects of organizers and their role in embryonal development. This motive rather than the intention of analyzing organismal differentials dominated a large series of such experiments. We shall analyze first, transplantations which were undertaken previous to the full development of the organizer concept, and then in a subsequent chapter we shall discuss transplantations which were carried out with the problem of organizers in view, as far as such experiments are of interest in the analysis of individuality.

The experiments of Lewis, Filatov and others, have shown that homoio- as well as heterotransplantation of skin can be readily carried out in amphibian larvae, and that in contact with the optic disc the transplant in either case is able to produce the lens of the eye. But the conditions under which the formation of the lens takes place vary in different species. In some species, such as Rana fusca, the skin from all regions of the organism retains up to a relatively late stage of development the ability to produce the lens in contact with the optic vesicle. In Rana esculenta, on the other hand, only the skin of the eye region is able to form the lens, though the transformation of the epithelium of the skin into lens apparently proceeds, through self-differentiation, independently of a previous contact with the optic disc. Skin from other areas is not able to produce lens in this species, but the optic disc has the same power to act as an organizer in contact with epidermis as that of other species. Bombinator behaves in a somewhat intermediate manner; certain areas of skin are able to produce lens tissue without contact with the eye vesicle, but the optic vesicle also has the power to induce lens formation in skin with which it is in contact. We have to deal in these cases probably with differences of a quantitative kind, and they seem to depend upon the stage of differentiation which the skin of the various species has attained at certain periods. In principle, there exists in all these species the potentiality of inde-

pendent transformation of skin into lens tissues, as well as the production of lens under the influence of the eye vesicle.

As to the length of time during which such homoio- and heterotransplants of skin remain alive, no systematic studies seem to have been made. However, that the heterodifferential may after all assert itself is indicated by the experiments of Filatov, in which larval skin of Bufo was grafted over the eye of Rana esculenta. The lens developed from the transplant but subsequently it degenerated. Perhaps a certain length of time was required for the cumulative action of the heterodifferential to become apparent.

Somewhat analogous conditions exist also in other instances. Thus, Ekman found that in larvae of Bombinator the ectoderm from the heart and kidney regions, but not from other parts of the body, if transplanted to the gill region is able to produce gills. There appears to exist a varying degree of rigidity of the tissue differentials in analogous tissues at corresponding periods of embryonal development in different species, and in addition, the tissue or organ differentials may be specialized to a different degree in different areas of the same individual.

Whether these differences in the degree of plasticity of the skin are in any way related to its transplantability and sensitiveness to strange organismal differentials seems not to have been determined. But it is quite obvious from these investigations that the tissue differentials may be graded in a much finer way than is apparent from the manifest structural characteristics of the tissues. That this is true also of tissues of the adult organism follows, for instance, from our studies of the varying ability of connective tissue in different parts of the sex tract to produce placenta.

In general, evidence is lacking that in transplantation in amphibian larvae the individuality differential plays any particular role. In urodele, as well as in anuran larvae, skin, extremities, tailbuds, eyes and other organs can be readily homoiotransplanted. However, under certain conditions individuality differentials may, after all, produce a certain effect; thus, according to Hellmich, in anuran larvae a homoiotransplanted limb may heal in, but subsequently the transplant, ceasing to grow, shrinks and becomes necrotic. Other effects of the individuality differential on transplantation in anuran larvae will be discussed later.

In urodele larvae heterotransplantation succeeds more readily than in anuran larvae. For instance, between Amblystoma punctatum and Amblystoma tigrinum extremities can be readily exchanged. Likewise, transplantation of extremities from larvae of Triton taeniatus to Salamandra maculata, and other similar heterotransplantations, may be successful. However, this is true only of transplantations in larvae. In metamorphosed urodeles, even after autotransplantation the transplanted limbs are readily cast off, an effect which must be due, however, to other factors than organismal differentials. Similarly when, according to Detwiler, autotransplantation of a limb in larvae of the urodele Amblystoma succeeds better than homoiotransplantation, this difference in all probability does not arise from the direct injurious influence of strange individuality differentials on the grafted tissue, but from secondary

effects, involved, perhaps, in the establishment of connections between the transplants and the central nervous system.

Gräper and Alverdes, on the other hand, find that transplantations of extremity buds from larvae of Rana palustris to Rana sylvatica succeed only temporarily; they retrogress within four to five weeks. Similarly, in earlier experiments Born had observed that transplantation of skin from larvae of Bombinator to Rana did not succeed very well. Likewise, Ekman had noticed that after exchange of gill ectoderm between these organisms the transplants were soon destroyed or cast off. Among amphibian larvae, and especially also in urodele larvae, the transplantations become more difficult if the species used are more distantly related. Thus, Harrison found that the balancer anlage can be readily transplanted from Amblystoma punctatum to Amblystoma tigrinum, but if this organ is grafted from Amblystoma punctatum to a larva of Rana sylvatica, only a short appendage develops. Similarly, the induction of the balancer in Triton by means of frog material gives only doubtful results (Mangold). An ear vesicle transplanted from a larva of Rana esculenta to Triton taeniatus remained alive at most for twenty-nine days, and in the majority of cases it disappeared even earlier; yet the transplanted organ, while it lived, was able to induce the formation of cartilage in the host (Balinski). In the tailbud stage of Amblystoma punctatum and tigrinum, pieces of spinal cord can be exchanged between these two species and may remain alive, the brachial plexus growing out from the transplant into the host. However, subsequently irregularities do develop; there is a greater mortality and metamorphosis does not take place in the bearers of the grafts (Wieman and Nussman). In urodele larvae heterotransplants from nearly related species may not only remain alive, but the tissues from both species may intermingle with each other, so that chimaerae develop. Thus Schaxel grafted parts of regenerative blastemas of extremities of white axolotls into autogenous blastemas of black axolotls, and vice versa. Although these tissues differed in their race differentials, they could be mixed in various ways without any resulting incompatibilities.

As to the manner in which unfavorable heterodifferentials may in the course of time injure the transplant, older observations of Braus gave no indications of differences between the results of auto- and homoiotransplantations of buds of extremities in anuran larvae; the transplants survived even through the period of metamorphosis, and extremities of host and donor metamorphosed at the same time. But, transplantations of buds of Bombinator larvae to larvae of Rana esculenta were only temporarily successful. The transplants began to develop and then, when a certain stage had been reached, growth and development ceased. Growth seems, thus, to be a more delicate indicator of incompatibility of organismal differentials than the life of the transplant; the former may stop under the influence of injurious factors of a heterogenous nature at a time when life still continues. As to the cause of cessation of growth at a certain stage, Braus believes that the critical time coincides with the period when the circulation is established in the host. This would suggest that heterotoxins are carried from the host to the transplant

by means of the circulation. However, we have already seen that incompatibilities between organismal differentials may become manifest even without injurious effects being transmitted through the blood. In Harrison's heterotransplantations of tails of anuran amphibians, atrophy and degeneration often set in within a few weeks after grafting, although some parts of the transplant could survive. But it is possible that vascular changes, interfering with the circulation of the blood, and caused presumably by the incompatibility of the organismal differentials of host and graft, were at least partly responsible for the degenerative conditions that occurred in the experiments of Braus.

It is of interest to compare with these transplantations of small pieces the results obtained in the joining together of larger parts of larvae of Rana esculenta and Bombinator. In Born's experiments such combinations lived only for three weeks, but, according to Braus, they may persist longer under favorable conditions. It seems, then, that in both cases after heterotransplantation incompatibilities developed, which caused a cessation of growth. We may conclude that certain, not well defined, growth factors may be potent even in heterotransplantations between amphibian larvae, and that the substances circulating in the body fluids of the host which regulate the growth processes, may be independent of organismal differentials, as are also other growth-regulating substances, such as certain hormones, which apparently do not carry organismal differentials. As in the case of tumors, we must distinguish from these hormone-like, growth-regulating substances, other growth-determining factors which are inherent in the transplanted tissue itself, and which continue to assert themselves even in a heterogenous soil, provided the heterotransplantation does not preclude the life of the transplanted tissues.

As to the relation of these inherent growth substances to the organismal differentials, these experiments do not give any indication. As stated above, between Amblystoma tigrinum and Amblystoma punctatum, limb, and also eye, can be readily exchanged, and both of these organs may then continue to live. Normally, Amblystoma tigrinum reaches a greater size than Amblystoma punctatum and the experiments of Twitty and Schwind indicate that the transplanted extremities retain essentially the characteristics, as to growth energy, of the species or race from which they are derived. This may perhaps be due to the fact that the growth factors inherent in the transplanted tissues dominate over extraneous growth factors, which are transmitted to them through the circulation of the host. Similarly, Burns and Burns found that heterotransplantation between larvae in these two species of Amblystoma succeeds if young stages are used for this purpose, and that under these conditions both partners retain their intrinsic growth momentum. Likewise, the specific stimulus to metamorphosis is not transmitted from one partner to the other, or if transmitted, is ineffective in these transplantations, but the sexual characters of one partner may be influenced by those of the other. Also, in the case of heterotransplantation of eyes the transplanted organ retains the growth energy inherent in the donor tissues. In some cases the inherent growth energy of the donor tissue may be the deciding factor only

in the first period of transplantation, while subsequently the transplant adapts itself to the growth rate of the host; this was observed after transplantation of the heart primordium from Amblystoma tigrinum to Amblystoma punctatum; moreover, here the more rapid growth of the transplant in the first period was accopanied by a more rapid differentiation.

On the other hand, it has been observed by Detwiler that when parts of spinal cord are transplanted from Amblystoma tigrinum to Amblystoma punctatum, the transplant not only grows better than the corresponding organs in the host tissue, but even better than the non-transplanted donor organ in Amblystoma tigrinum. Similar observations were made in the case of limb transplantations. It is possible that in this instance differences in the organismal differentials between host and transplant exerted a stimulating effect on the graft. However, this stimulation of growth following heterotransplantation of cord tissue again applies only to an early period; subsequently, an adaptation takes place between the size of the transplant and the corresponding organs in the host. The experiments of Detwiller regarding the factors regulating the growth of the nervous system prove that the outgrowth of nerves was not determined by species-specific substances.

The age of the host affects the transplant in a characteristic way. If an eye of a young organism is transplanted to an older host, its growth is accelerated, so that its stage of development after some time is equal to that of the host, while an older eye, having attained a more advanced stage of development, after transplantation to a younger host grows more slowly, so that the eye of the host, after some time, reaches the same stage of development as the transplant. Twitty explains these phenomena on the basis of Robb's specific partition coefficients for foodstuffs which different tissues possess, a theory related to the conception of athrepsia of Ehrlich. However, if differences in partition co-efficients, inherent in different tissues and changing in accordance with the ontogenetic stage of development, should be responsible for these results, this would presumably be a factor of only secondary importance, the primary factor consisting in differences in the inherent growth energy of various tissues, upon which would depend the amount of foodstuffs which the various tissues attract and use. The influence of age on the growth energy of the transplant appears to be similar to the effect which the time of metamorphosis has on the growth of transplants in urodele larvae and which will be discussed later.

In these more primitive organisms, such as larvae of urodele and anuran amphibia, there is some indication that relatively undifferentiated cells remain preserved through certain periods of larval life and that it is these cells which in ontogenetically more primitive organisms give origin to a blastema endowed with great regenerative potency. The presence of such cells would also account for the transformability of relatively primitive transplants under the influence of host tissues acting as organizers in a certain "action field" of the host. It may perhaps be assumed that in urodeles such less differentiated cells remain preserved longer than in anuran larvae and in this way the greater regenerative power of the former may be explained. These cells are,

as Hellmich points out, comparable to the totipotent cells which have been found in sponges (archeocytes), hydrozoa (interstitial cells), vermes (neoblasts), and tunicates (amoebocytes). Corresponding to the relatively undifferentiated character of such cells, their organismal differentials are presumably also as yet relatively little developed and they can therefore be successfully heterotransplanted, while this is impossible in ontogenetically further developed stages.

However, differences in the degree of differentiation of the organismal differentials do not depend merely on the presence or lack of certain undifferentiated cells, which, under ordinary circumstances, remain more or less dormant, but such differences must also exist in the ordinary tissues composing an embryo or a larva in various types of animals. It is presumably due to this inverse parallelism between the prospective potency of embryonal tissues and the degree of specificity of their organismal differentials, that in larvae of urodele amphibia extremities can be successfully homoiotransplanted under conditions which make such a result impossible in anuran larvae; in contrast with what is found in urodeles, in anuran larvae homoiotransplanted extremities placed in close proximity to a developing extremity of the host heal in only temporarily; they then cease to grow and undergo shrinking and necrosis. But so far a systematic comparison of auto- and homoiotransplantation of limbs has not, apparently, been undertaken, and this would be necessary before more definite statements can be made as to the development of organismal differentials in these embryos and as to the parallelism between the degree of organ and tissue differentiation and the fixity of the organismal differentials during different stages of embryonal life.

The stage of differentiation of the transplant seems to influence its fate in a graded way. We have seen that fully differentiated extremities of amphibia cannot be successfully transplanted into the skin or subcutaneous tissues. If somewhat younger extremities are used, only the less differentiated parts like perichondrium and other mesenchyme cells remain alive, become a part of the host and continue to differentiate. Also, after transplantation into the interior of the host the fully differentiated cells die and only the less differentiated cells remain alive. However, when very early stages of extremity buds are transplanted, although no further development takes place, necrosis does not occur and the mesenchyme cells of the transplant dissociate from one another and migrate into the host tissue (Hellmich); but in other cases, if an early embryonal bud is able to maintain itself in the host, it is more accessible to the organizer action of the surrounding host tissue than older tissues, and accordingly, it is modified in its development by the host tissue, whereas somewhat further differentiated transplants develop through self-differentiation. Correspondingly, several authors have stated that in transplantation of avian embryonal tissues the results are not favorable if very early stages are used, and similarly, Goetsch has observed that if in Hydra very early regenerative buds of the oral region are transplanted into the lateral zone of other polyps, the transplants are resorbed, whereas older re-

in the first period of transplantation, while subsequently the transplant adapts itself to the growth rate of the host; this was observed after transplantation of the heart primordium from Amblystoma tigrinum to Amblystoma punctatum; moreover, here the more rapid growth of the transplant in the first period was accopanied by a more rapid differentiation.

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The age of the host affects the transplant in a characteristic way. If an eye of a young organism is transplanted to an older host, its growth is accelerated, so that its stage of development after some time is equal to that of the host, while an older eye, having attained a more advanced stage of development, after transplantation to a younger host grows more slowly, so that the eye of the host, after some time, reaches the same stage of development as the transplant. Twitty explains these phenomena on the basis of Robb's specific partition coefficients for foodstuffs which different tissues possess, a theory related to the conception of athrepsia of Ehrlich. However, if differences in partition co-efficients, inherent in different tissues and changing in accordance with the ontogenetic stage of development, should be responsible for these results, this would presumably be a factor of only secondary importance, the primary factor consisting in differences in the inherent growth energy of various tissues, upon which would depend the amount of foodstuffs which the various tissues attract and use. The influence of age on the growth energy of the transplant appears to be similar to the effect which the time of metamorphosis has on the growth of transplants in urodele larvae and which will be discussed later.

In these more primitive organisms, such as larvae of urodele and anuran amphibia, there is some indication that relatively undifferentiated cells remain preserved through certain periods of larval life and that it is these cells which in ontogenetically more primitive organisms give origin to a blastema endowed with great regenerative potency. The presence of such cells would also account for the transformability of relatively primitive transplants under the influence of host tissues acting as organizers in a certain "action field" of the host. It may perhaps be assumed that in urodeles such less differentiated cells remain preserved longer than in anuran larvae and in this way the greater regenerative power of the former may be explained. These cells are,

plant by grafting embryonal tissues into the eye socket of amphibia after its contents had been removed. However, even under these conditions the transplant did not develop as a mosaic, but underwent various irregular transformations into other tissues, presumably under the influence of the host; thus ectoderm could differentiate here into neural or mesodermal parts, in contrast with what happened in salt solutions in vitro, where ectoderm merely proliferated and formed epidermis. This result applied to tissues transplanted at early stages of their development; if farther advanced tissues were used, the more fixed the organ differentials were at the time of transplantation, the more frequently normal organs were produced. Durken called this method "interplantation"; it represents a condition intermediate between the ordinary transplantation and tissue culture in vitro. Such experiments were carried out in anurans as well as in urodeles, but there was an interesting difference between the interplantation in the anuran Rana and the urodele Triton, in the former the homoiogenous material being toxic for the host. Such toxic effects, consisting in hemorrhages and necrosis, were not observed in case of syngenesiotransplantation. In urodeles, toxic effects were lacking. It is noteworthy that in these experiments individuals were apparently sensitive to toxic substances produced in their own species, although as a rule a high degree of immunity exists in a species against its own poisons. Kusche could successfully interplant early embryonal tissues which belonged to different genera in urodeles; for example, Triton cells continued to differentiate after transfer into larvae of Salamandra and Amblystoma; but if the interplantation took place between different orders, such as between Triton tissue and larvae of Rana fusca, or between cells of Amblystoma and larvae of Hyla, the interplants were destroyed. We may assume that under these conditions the bodyfluids of the host were toxic for the transplant, owing to the divergence between the organismal differentials in host and graft.

A number of investigators, beginning with Belogolowy, transplanted segmented eggs, blastulae, gastrulae or neurulae, into the peritoneal cavity of adult amphibia; or in other experiments the freshly fertilized eggs of Axolotls were transplanted into larvae of the same kind, measuring 13 to 15 mm. in length. The transplants were able in certain cases to undergo development, but this was abnormal, even after homoiotransplantation. However, if the relationship between host and transplant was very distant the transplants usually died. Belogolowy did not observe a difference between the results of homoiotransplantations and transplantations from Rana to Pelobates and vice versa; but transplants from Rana to Triton were found alive after one month only in exceptional cases.

In two instances Janda observed that in axolotls fertilized eggs, when transplanted to the peritoneal cavity of the same species, led, after three or four months, to the development of embryonal formations, which were very incomplete and structurally quite abnormal. Especially prominent in these formations were epidermal cysts, masses of cartilage and nerve tissue producing cysts; also, intestinal structures with glands and rudimentary kidney and eyes were found. As in Dürken's interplantations into the eye socket,

generative tissues can develop in accordance with the potentialities of the transplants. It seems, then, that too slight a differentiation of the transplanted tissue favors its complete adaptation to the host and is responsible for the lack of a growth momentum, which would otherwise have led the transplant to develop in its own way.

We shall consider transplantations in their relations to organismal and organ differentials still further in the following chapter, in which we analyze organizer actions and their connection with organismal differentials. But the data which we have already discussed lead to the conclusion that the outcome of transplantations in amphibia depends primarily upon two sets of constitutional factors, namely, (1) the phylogenetic stage of development; this, in urodeles, is more primitive, the precursors of the organismal differentials are, as yet, less differentiated and, therefore, the range of relationship in which transplantation of embryonal material succeeds is wider than it is in anuran amphibia. (2) The ontogenetic stage of development of the organismal differentials; the genes from which the organismal differentials develop are fixed for each species and individual, and they remain unchanged in all the tissues throughout embryonal development. In contrast to this fixity of the genes, the degree of differentiation which the organismal differentials have reached in successive stages of embryonal development differs; the greater the tissue and organ differentiation, the further advanced is also the differentiation of the organismal differentials, and correspondingly, the narrower will be the range of relationship within which the transplantations succeed. Besides, in the same embryonal stage different tissues and cells may differ as to the degree of differentiation they have attained, and, correspondingly, they may differ also as to the stage of transformation of the gene-precursors into the actual organismal differentials, which are localized in these tissues. Since organismal and also organ differentials are further differentiated and more fixed in anuran larvae than in urodeles at corresponding stages of embryonal development, the transplantability of the former will be less than that of the latter at any given period.

Furthermore, during regenerative newformation of limbs, conditions exist which in certain respects resemble those present during embryonal development (Schaxel); a graded differentiation of the regenerating tissues and a corresponding maturation of the organismal differentials take place. Therefore, if buds of extremities representing early stages of regeneration are transplanted, they may give origin to mixtures of various tissues; but if somewhat later stages are transplanted the tissues are fixed in their potentialities and in the localization of the regenerating material and under these conditions typical limbs develop. The great plasticity of the blastema of limb or tail of an amphibian is demonstrated in a remarkable experiment of Schotté, in which he showed that after transplantation into the eye of frog larvae, from which the lens had previously been removed, the blastema changed into lens tissue under the influence of organizers located in the eye of the host.

Dürken and Kusche diminished the effect of the host tissue on the trans-

Transplantation of embryonal material into the allantois of chick embryos. The first transplantations on the chorio-allantoic membrane of the chick were made with thin pieces of mammalian tumors, and these experiments indicated the great tolerance for heterogenous tissues which this organ exhibits. However, the chorio-allantoic membrane was used also for transplantation of embryonal material and a survival of the grafts was here observed when transplantation into the adult chicken would have been followed by the rapid destruction of the grafts. Thus Hoadley, Murray and others, found that sense organs and extremities of very young, one or a few days old chick embryos develop well, although apparently not always quite normally or completely, on the chorio-allantoic membrane of eight- to nine-day-old chick embryos. However, the time during which the transplants continue to grow under these conditions is not longer than about nine days; even heterotransplanted tisue can develop during this short period. Hiraiwa and Willier observed that parts of eleven-day-old rat embryos grew well for nine days on the chorioallantoic membrane of the chick; epidermis, hair follicles, cartilage and bone, were used and grew as well here as in rat embryos of the same age, but the entodermal and nerve structures did not continue to grow and differentiate in this strange host, or at least they developed less well. The age of the embryonal graft seems to influence the fate of the transplant to some extent. Sandstrom noted that kidney tissue from nine-day-old duck embryos healed in on the chick chorio-allantoic membrane without any part of it becoming necrotic. Older embryonal kidney tissue underwent partial necrosis and the necrotic areas persisted the longer in the host the older the embryo was from which the graft was taken; moreover, the activity of host lymphocytes also increased with increasing age of the grafted embryonal tissue.

The relatively favorable results of heterotransplantations on the chorio-allantoic membrane of the chick are probably due to the fact that the defense mechanisms, the sensitiveness to strange differentials of the host, and the organismal differentials, factors acting in combination or singly, are not yet fully developed in the placental structures of the early embryo.

Inoculation of mammalian embryonal material into adult mammals of the same species. Here a growth may take place, which at first may be quite rapid, but which then slows up, comes to a standstill, and finally is followed by retrogressive processes. In different species the tendency to active growth and persistence apparently varies. It seems to be very great in the rat, where the transplant may persist for months and even a year, although in most cases the proliferation may continue only from one to four months, when a cessation occurs followed by retrogression. The period of growth is relatively brief in the mouse, retrogression beginning usually after one week (Rous). In the rabbit, after transplantation into the uterus, it appears that only cartilage survives as long as twenty days (Hammond). However, in all of these experiments with homoiogenous tissues there is a great diversity in the results in different experiments, and even in the rat it is only in exceptional animals that the grafted tissues remain active for very long periods. It is

the surrounding host tissues did not show a marked activity. However, in the majority of cases the implanted material, it seems, was absorbed without having led to the formation of these embryomata.

In such transplantations, certain substances in the host may be toxic for the transplant, and, conversely, there is a tendency also for the hosts to be injured by the transplants and they may die within a few months. The toxic substances in the peritoneal cavity were found by A. Weber to be identical with substances present in the blood or lymph. Especially the eggs of Triton appear to be killed within a few minutes by such substances in an adult Triton. On the other hand, eggs of Bufo and Bombinator develop in the peritoneal cavity, or in the lymph sacs of adult animals of their own species, without being injured by toxins. But if Triton eggs, which would have died within five minutes in the peritoneal cavity of adult Tritons, are transplanted into the lymph sac of Bufo, they develop, although abnormally. Anuran eggs live for several hours in the peritoneal cavity of Triton. It seems, then, that it is particularly the eggs of Triton which are sensitive to these toxic substances.

It is not sufficiently clear in these experiments what role organismal differentials may play in producing such toxic effects. However, according to Weber the eggs survive longer in the peritoneal cavity of the parents than in homoiogenous individuals. While this indicates that organismal differentials may in some way be concerned in these processes, it is probable that essentially we have in these experiments to deal with the presence of special toxins, distinct from the heterodifferentials, which affect injuriously the transplants after ordinary transplantations.

Transplantation of avian embryonal tissues into adult birds. In the majority of experiments avian embryonal tissues were homoiotransplanted into the subcutaneous tissue, muscle of the chest, peritoneal cavity, or anterior chamber of the eye of adult birds (Féré, Wilms, Tiesenhausen and Skubisrewski). One-day-old embryos were not found suitable for transplantation; experiments with young embryos, about five days old, appeared most successful. The gross observation indicated that in the majority of cases the transplants did not grow, or growth disappeared within a month or two. In some instances, however, masses of irregularly arranged embryonal tissues, embryomata, developed, which grew to larger size and remained alive for a year or more; but in the end they also diminished again in size and were absorbed. Certain tissues, such as bone, cartilage, smooth muscle and squamous epithelium, were especially resistant. There was apparently no difference between the results, irrespective of whether the embryonal material was transplanted into the mother or into non-related individuals of the same species. However, considering the great variability in the results obtained in these experiments, definite conclusions cannot be drawn as to the effects of the relationship between transplant and host, although it seems that heterotransplantations, such as those of chick embryo to duck or pigeon, of duck embryo to chicken or pigeon, or also of mammalian embryo to chicken, resulted in an early degeneration of the transplants.

ing factors in these experiments. Similar effects may be observed also after homoiotransplantation of several pieces of adult mammalian tissue into the same host, as we have pointed out in a previous chapter.

In a relatively small number of experiments, various investigators transplanted embryonal material into the mother from which the embryo had been obtained; in some of these cases pregnancy continued, while in others it was interrupted. Freund found no difference in the rat between syngenesiotransplantation, that is, grafting of the embryo to its own mother, designated by this author as *autoplastic transplantation*, and homoiotransplantation, grafting of embryonal tissue to a strange host; but, considering the wide range of variations which has normally been found after homoiotransplantation of embryonal material, the number of experiments of this kind was not sufficiently large for definite conclusions. In contradistinction to Freund, Fichera noted that in rats the embryonal transplants persisted longer and more tissues developed in the own mother than in homoiogenous hosts. A similar result was obtained by Rous in the mouse. While the growth of the embryonal material was not more rapid in the mother than in favorable homoiogenous animals, in the former it persisted longer and led to the development of a greater variety of tissues. Thus it seems that even in the case of embryonal material the individuality differential, or its precursor, plays a certain role. Nicholas attempted to transplant embryonal limb or eye from brother to brother in the uterus, but technical difficulties made a successful transplantation only exceptional, and the effect of a close relationship between host and transplant in these experiments remained, therefore, uncertain. Likewise, reports as to the part played by pregnancy in the host on the fate of transplanted embryonal material are contradictory. Freund believed that such tissue grows better in pregnant than in non-pregnant hosts. However, according to Peyton Rous, in the mouse pregnancy inhibits the growth of transplanted embryonal material in a similar manner to that of tumor transplants.

Through repeated implantation of the embryonal tissue a relative immunity against the growth of subsequently inoculated embryonal tissue can readily be demonstrated, while in adult tissue such an immunity cannot be recognized with the same degree of definiteness. Both Peyton Rous and F. Fichera noted the development of this type of immunity; Rous produced a relative immunity in the mouse by means of a single inoculation of homoiogenous embryonal material, while Fichera made a series of injections of embryonal rat tissues into the adult rat; after each additional injection the immunity of the host became more pronounced. On the other hand, Paula Freund noted that a first unsuccessful subcutaneous inoculation of embryonal tissue in the rat did not need to prevent the growth of a second intraperitoneal graft. As in the case of active tumor immunity, we have not, in these experiments with embryonal tissue, to deal with a specific immunity to the particular homoiodifferential of the tissue which was used for immunization, since an immunity to all embryonal tissues of the same species seem to have resulted from the repeated injections. However, there is still the possibility that in

always cartilage, and, to a lesser extent, bone, osteoid tissue and bone marrow, and perhaps also smooth muscle tissue, squamous epithelium with its appendages, the hair follicles and sebaceous glands, which have the best chance to survive; to a lesser extent, entodermal (intestinal) and lymphoid tissue, and much more rarely, nerve tissue and rudimentary formations of sense organs, as well as glands such as liver, may be found in these embryomata.

Mammalian embryonal tissues give evidence of possessing species differentials and, consequently, do not withstand much better than adult tissues the injurious effects of heterotransplantation. Thus Rous observed that embryonal tissue of the mouse may grow in the rat for a few days, but then it dies. However, as to the individuality differential, there seems to be less selectiveness here, after homoiotransplantation, than in adult tissues; but this may be at least partly due to the fact that the original growth momentum is greater in embryonal than in adult tisues, and hence the former may be carried over some of the difficulties to which the latter succumb. Yet we find, also, in transplantation of embryonal tissues, great differences in the results obtained in different hosts of the same species. This indicates that individuality differentials may, after all, play a certain role in determining the fate likewise of the embryonal grafts. But in transplantation of mammalian tissue the age and developmental stage of the embryo, too, may be of some importance in the differentiation of the organismal differentials; in very early stages even species differentials are not yet fully developed.

That in transplantations of both normal adult and embryonal tissues the genetic relationship between transplant and host is a very essential factor in determining the outcome is further indicated by the fact that lymphocytic infiltration takes place around grafted living embryonal tissue. Two weeks after transplantation of embryonal tissue into the stomach wall of rats, Askanazy found an accumulation of lymphocytes around the transplant, and W. P. Neilson noted the same occurrence more recently in our laboratory. However, the interpretation of these observations is complicated by the fact that transplanted embryonal tissue not only grows, but also differentiates, and thus in the course of time becomes more like an adult tissue, and we cannot therefore be certain how much the maturation of the embryonal tissues had to do with the accumulation of lymphocytes.

Rous, in making two grafts of the same embryonal tissue into different places in the same host, observed that both transplants behaved in the same manner; either both did well or both retrogressed at an early date, or neither took, a finding analogous to ours in the case of tumors. This might be interpreted as indicating the importance of the individuality differentials of host and transplants in determining the result. But, Rous also found that mouse tumor tissue and mouse embryonal tissue transplanted simultaneously into the same mouse had a similar fate. Inasmuch as the individuality differentials of the embryonal and tumor grafts in this case were not identical, it appears that the sensitiveness and intensity of the reaction of a host towards strange individuality differentials in general likewise were determin-

However, while thus a great similarity exists between the successive changes in the phylogenetic and ontogenetic series as far as the development of the organismal and organ differentials is concerned, there are also some very important differences in these two series. In phylogenetic evolution we have to deal with changes in the genetic constitution of the organisms as the basis for the corresponding changes in organismal differentials and in individuality, and in differentiation and fixity of organs and tissues. On the whole, we can trace the relationship between different species of animals, one species developing from the other in an apparently connected series and each possessing its own kind of organismal differentials; the later species show a greater complexity and fixity in the organismal differentials than the preceding ones and, accordingly, also a greater differentiation and fixity of the organ and tissue differentials.

This same relationship obtains also if we compare the corresponding ontogenetic stages in the different classes and species of animals; but in ontogenetic development there is, as far as we know at present, in the consecutive stages of development throughout, an identity in the genetic constitution of cells and tissues in the same individual and species. And if in the series of ontogenetic stages likewise a development of the organismal differentials takes place, it is one from the precursor predifferential stage in the fertilized egg to the mature organismal differentials in the adult organism. The genetic basis of the organismal differentials remains unchanged in all these successive stages. We have then to deal, during embryonal life, with a development of the organismal differentials that is parallel to the development of organ and tissue differentials. In both, the genetic basis is already present in the egg, and notwithstanding this sameness of the genetic constitution throughout the series of successive ontogenetic stages, the constitution of the tissues as well as of the organismal differentials changes progressively. With *this increasing development of organismal differentials out of their* precursor stages or predifferentials, the mutual compatibility of tissues possessing different organismal differentials decreases. In testing by means of transplantation the effect of the increasing differentiation of tissues and organs and their differentials on the plasticity of organs, the adaptability of the organs and tissues to new environments, and the mutual interactions between the grafts and host tissues, we introduce at the same time a second variable in this experiment, namely, a change in the character of the organismal differentials which also have progressed in the direction from their precursors to more mature differentials in the course of embryonal development. In observing the effects of the new environment on organs and tissues we may thus have to deal with a summation of effects of both the changes in organ and in organismal differentials. It is conceivable that likewise during the process of regeneration in the earliest stages the precursors of organismal differentials are present in the regenerating tissues, and that with progressive regeneration the precursors of the organismal differentials present in ordinary tissues and in totipotent cells, mature into the fully adult organismal differen-

addition to this general reaction against homoiogenous tissue, a specific immunity against the particular individuality differential, which was used as antigen, may also have developed.

Rous believes that this acquired immunity manifests itself in the lack of a stroma reaction in the immunized mouse towards the transplant; an ingrowth of capillaries and connective tissue from the host into the transplant does not take place and the transplant dies as a result of this deficiency in blood supply. A lack of stroma reaction has also been observed by Rous in natural immunity, if a single transplantation of embryonal tissues is made into an unfavorable host. Rous does not mention the appearance of lymphocytes around the transplants in the immune hosts. A similar immune reaction has been described by Russell and Bashford in the case of grafted pieces of tumors, a mode of reaction which we shall discuss in a subsequent chapter.

If we wish to draw definite conclusions concerning the relations which exist between the state of the organismal differentials and the degree of differentiation and fixity of organs and tissues in various types of organisms, we again suffer from the difficulty that transplantations of embryonal material were not usually undertaken with this problem in mind. However, if allowance is made for a certain degree of inadequateness in the data, we may conclude that the range of transplantability in general is wider in the phylogenetically more primitive classes of animals, and that among the latter, in particular, it is wider in the more primitive urodele than in the anuran amphibia; that within certain limits in the ontogenetically earlier stages there is found both a lesser degree of organ and tissue differentiation and fixity and a lesser differentiation of the organismal differentials, and lastly, that the range of transplantability decreases with advancing embryonal development and differentiation. Furthermore, regenerating tissues in adult urodele amphibia have been shown to behave in certain respects like embryonal tissues; and corresponding to the increasing degree of organ and tissue differentiation, which is attained with advancing regeneration, transplantability of regenerating tissues likewise decreases. The earlier, less differentiated tissues are still more plastic and amenable to environmental factors, while the father advanced stages in embryonal development are more fixed in their organ and tissue differentials and thus have a greater tendency to develop by way of self-differentiation. This latter conclusion applies also to the regenerating tissue in the adult urodele. We find again, therefore, a relation between the differentiation and fixity of organ and tissue differentials on the one hand, and organismal differentials on the other hand, and in certain respects also a parallelism between the development of organ and tissue differentials and organismal differentials in the phylogenetic and in the ontogenetic series; but this parallelism exists only in a general way. As stated above, gradations in refinement of differentiation with advancing phylogenetic and ontogenetic development can be more clearly recognized in the case of organs and tissues than in the case of the organismal differentials.

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Chapter 5

Organizers and Tissue Differentiation, and Their Relation to Organismal Differentials

IN OUR DISCUSSION of the factors which cause organ formation in primitive organisms, we have referred to organizers localized in certain organs, which are able to induce the production of these same organs in another animal of the same species into which they have been transplanted. However, the "organizer" concept was not used originally in the analysis of organ formation in phylogenetically primitive species, but rather in embryos of less primitive organisms. The transplantation of pieces of organs may lead to the development of organs or of embryonal tissues other than those which function as organizers, and, in particular, the latter may induce the formation of parts of an organism normally adjoining the organizer tissue. It has been possible to trace this potentiality to the formation of organs and tissues and the distribution of organ-forming substances from the ovum through the first segments, through blastula and gastrula, to the more complex organisms. Associated with these changes is a parallel development of organismal differentials from their precursors, which also proceeds in the direction from less specific to more specific substances and mechanisms. It is this parallelism in these two processes and the possibility of a relationship between them which we wish to analyze in this chapter.

As stated, tissue and organ formation during embryonal life is brought about partly by substances which function as organizers in association with inherent, genetically determined characteristics of the tissues, which are the substratum on which the organizers act. The organizers may be defined as morphogenic contact substances, which serve as tissue transformers, or rather, as inductors, causing the tissues with which they come in contact to undergo certain changes, which, within a definite range of variability, are fixed by the constitution of the tissues upon which they act. In the earlier stages of embryonal development, when the plasticity and range of variability of the tissues are still very great, these substances may determine which of their potential differentiations the tissues will actually undergo. When in later stages the structure of the organism has become more stabilized, the organizers may exert quantitative rather than qualitative effects; they may determine not what kind of organs are to be produced, but what their size and position shall be and how many of them shall be formed; or they may stimulate the tissues to develop in a certain direction rather than to stand still or to undergo only relatively slight further differentiation. But this difference between the effects exerted in earlier and later embryonal stages is not a radical one; it is rather a difference of degree. As might be expected, during embryonal development we may have to deal not only with single inductions by or-

tials. This would not apply however to the highest organisms, where regenerative processes are much reduced in extent and where totipotent or even pluripotent cells no longer are present in the regenerating substratum.

In regard to the evolution of organismal differentials, and of the individuality which depends upon these differentials, there is, therefore, as far as we know at the present time, a radical difference between phylogenetic and ontogenetic development; and this difference is present notwithstanding the many structural and functional analogies which have been shown to exist between the various stages of phylogenetic and ontogenetic evolution, as far as the differentiation of tissues and organs is concerned. Furthermore, while the basic constitution of the organismal, and, in particular, also the individuality differentials corresponds closely to the genetic constitution of the various organisms, the subsequent differentiation of these organismal differentials depends not alone on these genetic complexes, but also on the progressive changes in organs and tissues which occur in the course of ontogenetic development within the same organism; a combination of both genetic and non-genetic factors is needed for the differentiation of the organismal differentials. We may assume that although both during phylogenetic and ontogenetic evolution a development and differentiation of the organismal differentials take place, the precursors of the organismal differentials must differ in these two series as widely as does the constitution of the egg protoplasm in a mammal and the cytoplasm in an ameba or in a coelenterate.

the same organizer may induce only quantitative changes in number, velocity and intensity of developmental processes. Furthermore, quantitative relations between the tissue acting as organizer and the tissue representing the substratum may play a certain role. Hence, if the substratum tissue is very extensive, it may offer an effective resistance to the activity of the organizer and appear inert; on the other hand, if the organizer tissue is very large, it may induce in the substratum changes which are greater from a quantitative point of view, although they may be of the same character as those effected by smaller pieces of organizer tissue. There seems to be active here, a quantitative relationship not unlike that characteristic of certain chemical interactions which determine the ultimate kind of equilibrium to be attained. We noted similar effects of the relative size of host and graft in transplantations in phylogenetically primitive organisms.

This struggle between the inductive activity of the organizer and the resistance of the substratum is also exemplified in the interaction between the organizer belonging to one species or order of animals and the substratum belonging to another species or order. In this case, the direction in which the differentiation of the affected tissue shall take place may be determined by the organismal differentials or their precursors in the substratum, rather than by the precursors of the organismal differentials in the organizer tissue. The organizer may transmit merely the impulse to further differentiation of the tissue in the direction of certain organ formations; but the character of these organs is modified by the characteristics of the species or order to which the substratum tissue belongs. The organismal differentials or their precursors do not exhibit a modifiability under the influence of organizers comparable to that which the specific substances of the various organs and tissues, the organ and tissue differentials, display.

Thus in the analysis of the organizer action use was made, especially by Spemann, Zeinitz and Schotté, of transplantations into different species and orders, either the organizer tissue being transplanted into a distant host, or the tissue serving as substratum being grafted into a different species in such a way that it came in contact with the organizer of the host. In both these instances the inductions expected took place. These experiments furnished at the same-time further data as to the transplantability of tissues representing early embryonal stages. It was in this way possible to graft successfully tissues belonging not only to different species, but even to different orders, and the latter type was called xenotransplantation. But in xenotransplantations there was sometimes noticeable on the part of the grafts a tendency not to enter into perfect union with the adjoining host tissue; however, the time during which the strange tissues were kept under observation in these experiments was short, because the main aim was the analysis of the organizers rather than of the organismal differentials or their precursors. Nevertheless, as far as such investigations make conclusions possible, they seem to confirm the view expressed in the preceding chapters, that in early embryonal tissues the organismal differentials, or rather the mechanisms through which their existence becomes manifest, are not yet fully developed and the range of

ganizers, but with chains of such transformations. An organizer induces a certain tissue and organ formation; there is associated with this transformation the production of a new organizer, which, on its part, induces a specific differentiation in the surrounding tissue and this again may lead to the formation of an additional organizer exerting a specific function.

There are, then, two sets of factors which fix the structure, chemical constitution, metabolism and function of tissues and organs: (1) The inherent characteristics of tissues and their range of modifiability, which may lead to development by "self-differentiation," and (2) the character of the organizers which are parts of the inner environment of the tissues. There is reason for assuming that, in general, without the action of the organizers the development of the early embryonal tissues would be very imperfect and rudimentary. This is indicated by the behavior of isolated embryonal tissues made to grow in vitro; even here, differentiation of tissues seems to depend largely upon the interaction between adjoining tissues and their organizers, and if this interaction is lacking, further differentiation does not take place. However, the artificial growth stimuli as such, acting in vitro, tend to prevent further differentiation. The less evident the action of the organizers is in the differentiation of tissues, the more the tissues appear to develop as the result of inherent conditions by way of self-differentiation. Development by self-differentiation usually leads to a more restricted formation of organs than that which takes place under the influence of organizers. In general, with advancing differentiation and increasing fixity of tissues in the course of embryonal life, self-differentiation comes to play a greater role and the tissue will depend less upon specific environmental organizer effects.

Furthermore, with the increasing development and differentiation of the organizer tissue, the organizer may change or the organizer effect may be lost, although even tissues in an advanced stage of differentiation, such as retina or brain, may still exert some of the organizer action which the more primitive precursors of these tissues exerted. Likewise, with increasing differentiation, different parts of the organizer tissue may begin to undergo modifications in respect to the organizer functions they exert.

Both organizer and conditions inherent in the recipient tissue or substratum are then of importance in embryonal development; therefore a tissue may develop by self-differentiation in the absence of an organizer and it may be modified in its development by the presence of an organizer. Of course, there may be always hidden in the apparent process of self-differentiation some previously exerted effects of organizers. Now, this interaction between these two sets of factors may assume the character of a competitive struggle between the inertia of the substratum, with its varying potentialities, and the inductive activity of the organizers. Thus the same organizer may be able to accomplish a certain transformation with one tissue with which it comes in contact, but not with another. Or the difference may be of a quantitative character rather than an absolute one; the organizer may be able to induce a certain change more readily in one organ than in another; or in one tissue the organizer may readily induce newformation of a certain kind, while in another

However, the injured or killed material does not behave exactly like living tissues from the same or related species or orders of animals; the former seems to be less specific, as shown by the fact that it induces a smaller number of transformations in the tissues on which it acts, and the fine differences between different parts of a certain organ used as organizer are lost under these conditions. Thus, while in the normal medullary plate of Triton different portions are differentiated—the anterior portion inducing formation of brain, eyes, nose, ear vesicles and balancer, the posterior portion inducing formation of spinal cord and tail—in the medullary plate produced by killed material these specific differences between anterior and posterior portions are no longer present, the different parts acting alike. It is especially the development of neural tube from ectoderm of the gastrula which can be induced by dead organizer material. The production of certain mesodermal structures, such as kidney, musculature, bone and extremities, can only barely be initiated by killed organizer tissue, and at best these organs and tissues are formed only in small quantities. But coagulated embryo extract of the chick may call forth not only the development of nerve tissue in gastrula ectoderm, but even of chorda and musculature. It seems, after all, that there is no absolute, but only a graded difference in the ability of dead tissue to function as organizer and in the specificity of the transformations brought about by it, as compared with the effects of living tissues of the same kind. Needham and Waddington distinguish two types of actions of organizers: (1) The organizer reproduces or tends to reproduce the axis of the embryo and, ultimately, a more or less whole, early embryonal stage of the organism, which furnishes the substratum for its operation. This process is designated as "evocation" and the organizer involved is called an "evocator." It is a relatively non-specific action, which may be shown also by dead material, and it represents a much more simple chemical effect than that exerted by (2) the individuator which produces certain subdivisions of the axis. The latter type of action is exhibited only by living tissue. Somewhat related views have also been expressed by Weiss.

The ability of xenoplastic tissues to act as organizers suggests that the organizers are either entirely devoid of organismal differentials, or bear organismal differentials with a very slight degree of differentiation. This enables the transplanted tissues which contain the organizer to exert their function in the host, notwithstanding the great difference in organismal differentials, or their precursors, in host and transplant; likewise, the experiments with dead organizer material suggest that the organizers, at least those possessing the more restricted, the evocator functions of the killed tissues, are devoid of organismal differentials. In contrast to the lack of organismal differentials in the inducting and transforming substances, the living substratum on which they act does possess organismal differentials.

We may enlarge somewhat on these more general statements by citing some specific experiments. If we transplant prospective medullary plate into regions where the ectoderm normally develops into epidermis, the transplant may, in its new location, merely form skin. Conversely, prospective epidermis

transplantability in these early embryonal states is, therefore, still greater than in the later ones.

While it was possible to keep embryonal tissues alive, at least for a short time, after hetero- or xenotransplantation and to obtain organizer effects, it has been found more recently that it is not necessary to transplant living tissue in order to obtain induction. In these experiments the dorsal lip of the blastopore from urodele gastrulae, which had been found to contain especially efficient organizer material, was used primarily, but also other material, such as medullary plate, with the underlying mesodermal structures, showed marked organizer effects. In contact with ectoderm of the gastrula, this material caused the transformation of the ectoderm into a medullary plate. Thus Spemann, Bautzmann, Holtfreter and Mangold could show that tissue which had been killed by exposure to heat or cold, by drying or by mechanical means, could still function as an active organizer. Likewise tissues treated with alcohol, ether, acetone, glacial acetic, hydrochloric acid or xylol, or infiltrated with paraffine, were still effective. Moreover, tissues which, in the living state, lacked the ability to act as inductors, acquired this property after they had been killed by drying (Holtfreter), or following treatment with acetone and alcohol. Thus entoderm or ectoderm of gastrulae acquired the ability to induce medullary plate formation after they had been exposed to such treatment. Even the unsegmented egg could, under these conditions, induce the development of very differentiated organs, such as the lens of the eye, whole eyes, optic vesicles, and parts of the brain. Spemann has suggested that the manifestation of organizer action in material formerly devoid of such effects may be due to the removal, by means of solvents, of inhibiting substances which had been present in the living, inactive tissues, or the changes which take place during the denaturation of protein may set free the active organizer. As to the chemical nature of the substances which act as organizers, the evidence obtained so far appears to be contradictory; the effects have been attributed to various substances, glycogen, proteins, and simpler hydrocarbons. It is possible that proteins in combination with glycogen or with certain non-specific lipoid substances may act as organizers; also estrogens and carcinogenic hydrocarbons may perhaps exert organizer function (Needham and Waddington). Some investigators have found that injury of embryonal tissue may activate the organizer.

Considering the fact that dead tissues of amphibian embryos and certain extracts from such tissues may serve as organizers, it is not surprising to learn further that a great number of organs of embryos or adult forms of vertebrates as well as of invertebrates may act as organizers in gastrulae of Triton. In some instances adult tissue from distant species has first to be killed by heat before it will thus act; but in other cases, strange adult living tissues exert this function after transplantation.

These results apparently are contradictory to the great specificity of the factors which are evident during embryonal life, where only definite organs and not others can act as organizers at given periods of embryonal development and in definite areas of the embryo if certain results are to be obtained.

epidermis, may be transformed into mesoderm if it is transplanted into a place in the embryo where normally mesodermal structures develop. After further transplantation, these mesodermal tissues, if they are brought into contact with other ectoderm, are able to induce the formation of a medullary plate, and the medullary plate can induce the formation of medullary plate and other nerve structures from ectoderm.

As stated above, in addition to organizer actions induced from the outside, there are active processes inherent in the tissues themselves, leading to self-differentiation; during normal embryonal development these two processes seem to cooperate in various combinations, in which the relative importance of each factor may differ quantitatively. Various kinds of interaction may thus be produced experimentally. We have referred previously to an instance in which an organ, although much differentiated, still retains its ability to act as an organizer. The optic disc in certain stages of embryonal development can induce lens formation in some species only in the cephalic ectoderm, while in other species at a certain stage of differentiation, also ectoderm of the rest of the body can be made to develop into lens. Now, Mangold has found that the eye-forming substances are determined in the ectoderm a short time after the mesodermal tissues and the chorda, constituting the roof of the archenteron, have formed and have been able to act on the ectoderm; it is therefore possible that this contact induces the ability of the overlying ectoderm to differentiate into an eye. However that may be, it can be shown that, from a certain period in embryonal development on, there is manifested in the overlying neural plate, as a result of increasing self-differentiation, an inherent tendency to produce optic vesicles independently of any organizer action. Yet even then, according to Adelman, an organizer action may be associated in its effects with this process of self-differentiation. The roof of the archenteron of Amblystoma not only tends to reinforce the inherent tendency of the ectoderm to form eyes, but it also modifies the place in the neural plate where the eyes develop. Inherently the median portion of the neural plate has a greater tendency to form eyes by self-differentiation than the lateral parts; but the underlying roof of the archenteron acquires a marked bilateral polarity in the course of embryonal development, and this condition influences the organizer action of this tissue; the lateral parts of the underlying tissue now gain a greater tendency to induce or to intensify eye formation than its median part, the organizer action dominating over the forces inherent in the neural plates and causing the production of lateral eyes.

These interferences between self-differentiation tendencies and organizer action can be shown in still another way. If the lateral parts of the neural plates are transplanted together with the underlying organizer tissues, more eyes are formed than would develop without the latter. If, on the other hand, the median parts of the neural plate are transplanted without the underlying tissues, they form eyes just as well; however, median parts of the underlying tissue, when transplanted with the median neural plate, frequently cause the separation of the eye-forming material into two eyes, while without this tissue, more often only one eye forms. In this case the organizer exerts effects

transplanted into a defect in the region of the presumptive medullary plate, is able to develop into parts of the central nervous system and it may give rise to the formation of an eye. On the other hand, if a differentiated medullary plate, together with adjoining mesodermal tissue, which will give rise to chorda formation, is transplanted into a gastrula, it may induce in the overlying ectoderm the formation of a medullary plate. We see, then, that an organizer may induce in the recipient tissue either structures of the same kind as the organized tissue, a process we may designate as "isoinduction," or it may induce structures of another kind, "alloinduction," as when archenteron induces formation of medullary plates. Similar differences in organizer action we mentioned in our analysis of the factors which are potent in transplantation in lower adult vertebrates.

The isoinduction which we mentioned, may be of a very specific character. As Mangold has shown, the medullary plate of the neurula may be divided into four parts in the direction from head to tail, and each part is then found to induce in the host the formation of those organs into which that particular segment of the medulla itself would have developed, although to some extent the effects of the different segments are overlapping. However, the various parts of the underlying mesodermal tissue may also exert corresponding specific formative effects, cephalic parts of the roof of the archenteron tending to induce the nervous structures characteristic of the head, the posterior portions tending to induce the tail parts.

While in some cases the ability of a tissue to act as an organizer may be retained with further development, or may become specifically localized in certain portions of the organizer area, in other cases this power is lost. Thus not only the medullary plate, but also the fully developed brain tissue may function as an organizer for the transformation of ectoderm into medullary plate. Similarly, the embryonal optic disc, as stated above, can call forth in the overlying ectoderm the formation of lens tissue. With further differentiation of the optic disc, such action has apparently been transferred to the fully differentiated retina of the Triton eye, which now has gained the power to induce in the iris of the eye the formation of a lens. On the other hand, under certain conditions, with increasing differentiation a diminution or a specific limitation in the capacity to serve as an organizer may be noted. From the dorsal lip of the Triton gastrula, which, as we have seen, acts as a very effective organizer, there develops chorda as well as mesodermal structures; but Mangold has demonstrated that it is only the chorda which preserves for some time the ability to induce the production of a medullary plate from the presumptive epidermis, while the mesodermal structures have lost this function.

During embryonal development we may have to deal with chain reactions induced by successive organizers. Certain mesodermal structures may induce the formation of the optic disc, and the optic disc in contact with ectoderm induces the formation of a lens; but here the chain reaction ends, the lens not being able further to act as an organizer. Another chain reaction is the following: ectoderm, which under normal conditions would differentiate into

recipient transplanted tissue, although the latter belongs to a different order. While, therefore, the organismal differentials inherent in the ectoderm of Hyla influnced the type of organ which would develop, the organizer modified the size and cell number in the developing organ.

We have seen that in early phylogenetic stages the character of the organismal differentials carried by the parts which are joined together is of importance in determining the mode of interaction between the different tissues of the two partners. Similarly, we may inquire how a gastrula and a piece of tissue to be grafted into it, one or the other of which is the carrier of an effective organizer, will interact when the transplantations are of a heterogenous or xenogenous character.

Some important observations which have a bearing on this problem were made many years ago by Lewis, who found that the optic vesicle was able to induce in embryonal skin the production of cornea, even if skin and optic vesicle belonged to different species. Spemann transplanted ectoderm, representing presumptive abdominal skin, from Triton taeniatus into the anterior, the brain portion of the developing nervous system of Triton cristatus. The latter acted as inductor and transformed the skin into central nervous tissue, which retained, however, the original species characteristics of Triton taeniatus. Similarly, after heterotransplantation of ectodermal tissues from Triton taeniatus into the gill region of Triton cristatus, the gill which developed from the taeniatus tissue under the influence of cristatus organizers retained the characteristics of the taeniatus species.

More recently, Mangold showed that the mesodermal structures and chorda of the host could act as organizers even towards heterogenous presumptive ectoderm transplanted into different species of Triton (cristatus, alpestris and taeniatus). The differentiation of the transplant under the influence of the heterogenous organizer tissue proceeded in the same way as after homoiotransplantation; the rudimentary embryos consisted of constituents of two different species, which, uniting harmoniously, thus represented chimaeras. More extensive investigations of organizer functions following transplantation into different species with varying degrees of relationship were made, especially by Zeinitz, Bytinski-Salz, and Schotté. While in the majority of such experiments tissues from different species were transplanted into Triton taeniatus, Bytinski-Salz carried out also the reciprocal transplantation of parts of Triton taeniatus into a number of nearly or distantly related species. In these experiments either the organizer tissue was transplanted into the bearer of the recipient substratum, or the recipient tissue of a strange organism was transplanted into the carrier of the organizer tissue. If in this way tissues from a more distant species were made to act on each other, difficulties arose in certain instances, although even after transplantation of anuran tissues into Triton, organizer effects could occasionally be observed.

Whether under such conditions an organizer effect occurs depends essentially upon three sets of factors: (1) The relationship between host and transplant and the influence of organismal differentials; (2) the effect of toxic substances. While to a certain extent the degree of toxicity may be influenced

of a quantitative rather than of a qualitative character. We have here to deal, as in all vital phenomena, with a quantitatively varying interaction between genetically determined, inherent factors and inner or outer environmental factors. It must be borne in mind that inner environmental factors may also be genetically determined.

The relations between inductor and recipient substratum may vary in different cases. As Mangold points out, ordinarily an organizer transforms the substratum in such a way that both organizer and substratum form one whole, which tends to reproduce the organism in which this transformation occurs. This condition Mangold designates as "complementary induction." But, in other cases the organizer gives rise in the host tissue to the formation of structures which do not fit into such an organization, as when double or other abnormal structures develop. Such an occurrence Mangold calls "autonomous induction." This is found only under abnormal conditions; for instance, when the age and stage of differentiation differ very much in inductor and substratum, so that the typical sequence of interactions is disturbed. Or, in other cases an autonomous induction may take place in case of xenoplastic transplantation when the organismal differentials of host and transplant are so strange to each other that a complementary result becomes impossible.

These observations present an interesting parallelism to those found after transplantation of adult pieces of organs or tissues in primitive classes of animals. Here also the transplant may unite with the host in an integrated manner, leading in the end to the formation of a normal individual. We may assume that under these conditions a tissue acting as organizer causes complementary induction; but if the surfaces of contact do not fit each other, or if the organismal differentials are too far removed from each other, then an autonomous induction takes place in the embryo, while in the adult the transplant becomes absorbed or is cast off and the host tissue may undergo regenerative growth processes.

As to the effect of the relative size of organizer and recipient tissue, Bytinski-Salz has observed that within a certain range the larger the organizer piece the greater its effectiveness, other conditions being equal. Quite recently Schotté carried out some experiments which, while made for other purposes, also have some bearing on this question. He transplanted large portions of the ectoderm, including the presumptive medullary plate, from young gastrulae of Hyla crucifer to the face region of Amblystoma punctatum, the former a relatively small and the latter a relatively large organism. Under the influence of the large quantity of organizer for mouth organs which was present in the face region of the Amblystoma host, mouth organs formed in Hyla skin, which were typical suckers with Hyla character, but they were three times as large as they normally are in Hyla. The number of cells which entered into these suckers was likewise about three times greater than is normally observed in Hyla embryos. Similar results were obtained with the induction of other organs, such as lenses, nasal placodes, ear vesicles and mouth organs. We may assume that the larger quantity of organizer material present in certain regions of Amblystoma induces formation of correspondingly larger organs in the

An organizer effect in case of xenotransplantation may even be demonstrable if the organizer action takes place at somewhat later stages of embryonal development. Thus the ectoderm of the gill area can, to a certain extent, transform ectoderm from other than the gill region of a xenogenous gastrula into gill tissue.

We may then conclude that the organizer can, in some instances, continue to function, but usually only in a very limited way, if very distant organismal differentials interact with each other. Moreover, it can be shown that also the factors which determine self-differentiation leading to further development may still act after xenotransplantation of an embryonal piece of tissue. Thus, according to Bytinski-Salz, anuran presumptive mesoderm after xenotransplantation, may differentiate into chorda and musculature, presumptive epidermis into epithelium of the skin.

Mangold and Seidel succeeded in joining together early stages of segmentation of Triton eggs belonging to the same species; in some cases a single homoiogenous organism resulted from this combination, in other cases two or more organisms developed. Mangold found that also union of heterogenous Triton eggs in the two-cell stage of segmentation may succeed, but the number of single organisms which resulted was smaller than after homoiotransplantation. After heterogenous union various organs which developed could contain constituents of both species, which functioned without any antagonistic reactions becoming manifest. However, as stated above, even under these conditions various abnormalities developed in the case of chorda. It is these abnormalities observed in heterogenous early embryonal combinations, which suggest that noticeable differences exist also in the character of the precursors of heterogenous organismal differentials, and although such differences usually do not become evident, they may lead to incompatibilities under certain unfavorable conditions.

In combining heterogenous parts in adult individuals belonging to different species in primitive classes of invertebrates, we have noticed that it is usually the larger piece which dominates over the smaller piece. Similarly after transplantation of small parts of embryos it is the larger host which is usually the dominating partner, the xenotransplant being, in most cases, either discarded or destroyed; but if the transplant belongs to a species particularly toxic for the host, the latter may be injured and ultimately killed by the transplant.

If thus xenogenous transplantations may succeed in amphibia and organizer effects be exerted, these effects become manifest after a relatively short time of interaction between the two strange tissues, a period too short perhaps for the manifestation of incompatibilities between the organismal differentials.

We have already referred to experiments of Spemann which showed that under the influence of heterogenous inductors the receptive tissues undergo differentiations into organs which are in accordance with the specific organ-forming potencies of the organizers; yet at the same time the organs and tissues which do develop show the species characteristics of the recipient tissues.

Some very instructive experiments of a similar nature, illustrating the

by the taxonomic relationship between host and transplant, the correspondence between toxicity and taxonomic relationship is not perfect. There are some species which are especially poisonous for Triton, although they may not be farther removed in relationship than other less poisonous species. (3) The relative rapidity of embryonal development in host and transplant. If in the transplant the development takes place very rapidly, then sufficient time may not be available for the organizer action to become effective.

In general, under the best of conditions xenogenous transplantations are difficult. In many cases the transplant is expelled or absorbed after it has been lying for some time in the deeper parts of the host as an apparently inert, though living tissue; but it may be possible to transplant presumptive epidermis of Bombinator into the ectoderm of Triton and have it develop here into skin; but such skin soon becomes thinner and gradually it disappears. After transplantation into deeper parts of the host it is either discarded or gradually absorbed, although in this position the graft may at first act like an interplant. For a short time it may develop and possibly even be effected by the organizers of the host, producing mesodermal structures. But union does not take place between host and transplant and chimaerae do not form.

If presumptive medullary plate is transplanted from Bombinator into Triton, two medullary tubes may develop in the host; one is from the transplant itself, the other, originating in the host, is determined by the transplant acting as an organizer. But under these conditions interesting differences may appear in the further fate of these newformations, from those observed after heterotransplantation, owing to incompatibilities between the organismal differentials of the two tissues. While the medullary tubes arising after heterotransplantation may coalesce into one single organ, those arising after xenotransplantation are unable to do so; at best there may be a temporary union between the two medullary formations, which is followed secondarily by a separation. A difference between the behavior of hetero- and xenogenous structures was found also in mesodermal formations. Here the difficulties in the union of xenogenous structures may be still greater than those observed in ectodermal tissues. While two medullary plates or tubes of xenogenous origin may exist side by side, with mesodermal tissues single organs develop either from the host or from the transplant, the one which develops first apparently suppressing the other. In the case of mesodermal chordae, heterotransplanted organs may not unite with the analogous host organs. Here we find, then, incompatibilities similar to those which are observed in the union of distantly related eggs or young embryos in echinidae and Ascaris, or in heterotransplantations in adult hydrozoa and planarians. In all these instances the character of contact mechanisms, which presumably is contingent upon the mutual suitability of contact substances, primarily determines the possibility and durability of coalescence. As we have seen previously, the completeness of the union depends, at least in some instances, upon the lack of regenerative growth processes at or near the point of contact, and this again is determined by the relationship of the two organismal differentials which interact.

direction. The more anteriorly a tissue is situated, the greater is the variety of tissues which it is able to produce; in the posterior direction the frequency and completeness in the production of such a variety of tissue are, step by step, decreased. We have evidently to deal with a multiplicity of factors which determine the formation of these structures and which also bring about in the course of embryonal development a gradually diminishing receptiveness of the tissues to the stimuli of the organizers.

In some respects we observe here, in principle, the same conditions which we found in the cervix of the guinea pig, where there is a gradual decrease in the potency of the tissues in one direction to form uterine structures, and in the other direction to form vaginal structures under the influence of hormones. We may consider uterus and vagina as representing two opposite poles. In passing from one pole to the other, or in the opposite direction, there is a graded change in structure and in mode of reaction to hormones.

Thus it is seen that there is a close correspondence between the action of organizers and that of well known hormones, which occur in invertebrates as well as in vertebrates, but which have best been studied in mammals. The organizers represent hormones which are present and act locally in contact with the recipient tissue, in contradistinction to distance hormones, which act after being carried to a distant recipient organ; the former are contact hormones produced in the cells and causing cytoplasmic differentiations in certain responsive tissues with which they are in contact. These organizers are devoid of the finer organismal differentials and there are indications that they may not possess any organismal differentials.

Further instances of correspondence in the action of contact and distance substances may be cited: In the case of the corpus luteum it has been shown, in the guinea pig, that a very interesting correlation exists between the time during which the hormone produces the maximum effect on the recipient tissue, namely, the uterine mucosa, and the period during which such a hormone effect is needed for the embedding of the fertilized ovum. It is only at a time when the hormone is produced in full strength that the tissue exhibits its full responsiveness. After the period has passed during which the egg normally attaches itself, the recipient tissue loses its responsiveness to less specific stimuli to which it was formerly responsive, presumably because the quantity of hormone necessary for sensitization of the tissue is diminished, or because a refractory state develops in the uterine mucosa.

A somewhat similar condition exists in the relation between organizer and recipient embryonal tissue. Here, as Lehmann has pointed out, the time during which the organizer is produced in maximal quantity in the upper lip of the gastrula of a certain species corresponds to the time when the ectoderm of the gastrula, which is the recipient embryonal tissue, is responsive to the action of the organizer. This correspondence applies, however, only if organizer and recipient tissue belong to the same species; it does not apply if organizer and recipient tissue are derived from distantly related species; in the latter case, abnormalities may result.

There are, however, other cases in which a hormone is still produced at a

specific effects of the organismal differentials of the recipient tissue, were reported by Schotté. He transplanted presumptive skin from the abdomen of anuran Rana or Bombinator gastrula into the mouth region of urodele Triton or Amblystoma embryos. In the transplants there developed mouth organs under the influence of the organizers of Triton and Amblystoma; however, whereas in the latter species a balancer would have formed, in the anuran transplants anuran mouth organs, such as suckers, horny jaws and teeth, as well as operculum, developed, each one in its characteristic place. We must therefore conclude that the organizers in the mouth organs of Triton or Amblystoma tend to induce amphibian mouth organs in general, but not the specific urodele mouth organs. The character of the recipient tissues, and in particular the characteristics determined by the organismal differentials or their precursors inherent in the transplant, determine what species characteristics these organs shall possess. It is, of course, possible, although not very probable, that in addition to the organizer substances, other less specific factors localized and inherent in the mouth region, participate in bringing about this result.

These findings again show the intimate connection which exists between the organizers, whose functioning leads to the development of specific tissues and organs, and the organismal differentials. A similar connection was noted in the case of inductions produced in the transplant by the host tissues, or in the host tissues by the transplant, in phylogenetically primitive classes of animals. Here, also, we observed that the species characteristics of the strange tissues were fixed, but that the determination of the kind of organ which was to develop was influenced by the inducting substances which asserted themselves, notwithstanding the strangeness of the organismal differentials.

While we have so far reviewed only experiments in amphibia, in principle, similar conclusions hold good also in other classes of animals. Thus in the chick embryo at the stage of the primitive streak formation, the potentiality of embryonal parts to form various tissues and organs is greater than is indicated by the tissues and organs which actually are produced during normal embryonal development. This fact has been established by means of transplantation of parts of the embryo into the chorio-allantois of the chick embryo. In this way it has been found, for instance, that heart can be produced at three different levels, and gut may develop from all levels of the primitive streak. The portion anterior to the pit can produce liver and mesonephros and the portion posterior to the pit can produce adrenal (Hunt). In the normal embryo substances are presumably given off by tissues, which inhibit the development of certain neighboring tissues and organs in a similar manner to that noted in the two-cell stage of echinoderm eggs, when one blastomere inhibits the other from developing into a whole embryo. But other tissues which normally develop in the embryo in a certain place, may not develop if isolated parts of the embryos are transplanted, perhaps because under the conditions of isolation needed organizer substances may be lacking. Furthermore, we may assume that the ability of the embryo to form tissues varies in the direction from the oral to the aboral pole of the primitive streak and also in a lateral

direction. The more anteriorly a tissue is situated, the greater is the variety of tissues which it is able to produce; in the posterior direction the frequency and completeness in the production of such a variety of tissue are, step by step, decreased. We have evidently to deal with a multiplicity of factors which determine the formation of these structures and which also bring about in the course of embryonal development a gradually diminishing receptiveness of the tissues to the stimuli of the organizers.

In some respects we observe here, in principle, the same conditions which we found in the cervix of the guinea pig, where there is a gradual decrease in the potency of the tissues in one direction to form uterine structures, and in the other direction to form vaginal structures under the influence of hormones. We may consider uterus and vagina as representing two opposite poles. In passing from one pole to the other, or in the opposite direction, there is a graded change in structure and in mode of reaction to hormones.

Thus it is seen that there is a close correspondence between the action of organizers and that of well known hormones, which occur in invertebrates as well as in vertebrates, but which have best been studied in mammals. The organizers represent hormones which are present and act locally in contact with the recipient tissue, in contradistinction to distance hormones, which act after being carried to a distant recipient organ; the former are contact hormones produced in the cells and causing cytoplasmic differentiations in certain responsive tissues with which they are in contact. These organizers are devoid of the finer organismal differentials and there are indications that they may not possess any organismal differentials.

Further instances of correspondence in the action of contact and distance substances may be cited: In the case of the corpus luteum it has been shown, in the guinea pig, that a very interesting correlation exists between the time during which the hormone produces the maximum effect on the recipient tissue, namely, the uterine mucosa, and the period during which such a hormone effect is needed for the embedding of the fertilized ovum. It is only at a time when the hormone is produced in full strength that the tissue exhibits its full responsiveness. After the period has passed during which the egg normally attaches itself, the recipient tissue loses its responsiveness to less specific stimuli to which it was formerly responsive, presumably because the quantity of hormone necessary for sensitization of the tissue is diminished, or because a refractory state develops in the uterine mucosa.

A somewhat similar condition exists in the relation between organizer and recipient embryonal tissue. Here, as Lehmann has pointed out, the time during which the organizer is produced in maximal quantity in the upper lip of the gastrula of a certain species corresponds to the time when the ectoderm of the gastrula, which is the recipient embryonal tissue, is responsive to the action of the organizer. This correspondence applies, however, only if organizer and recipient tissue belong to the same species; it does not apply if organizer and recipient tissue are derived from distantly related species; in the latter case, abnormalities may result.

There are, however, other cases in which a hormone is still produced at a

time when the recipient tissue has already lost its ability to interact with this hormone. Thus the anterior pituitary may continue to produce follicle-stimulating hormone in old persons, at a time when the ovary no longer possesses the structures which are able to react with this hormone. In a comparable manner, according to Mangold, the epidermis of axolotls is unable to respond with the production of a balancer at a time when the adequate organizer is present in the archenteron and medullary plate; but in other urodeles the recipient organ may actively respond to the presence of this organizer.

In recent years it has been discovered that there are hormones which mediate some effects of genes on those tissues which are under the control of these genes (Kühn, Ephrussi, Beadle). Such hormones develop, therefore, not under the influence of cytoplasmic, but of nuclear constituents. They may transmit to distant places, for instance, the effects of genes which distinguish the dominant characteristic of a wild race from the recessive characteristics of a mutant race. These gene-hormones have been found in various orders of insects, such as Ephestia, Bombyx, Habrobracon and Drosophila; they may occur in certain organs (ovary, testis, brain), or in the bodyfluids, and they can be conveyed to other organisms either by implantation of these organs or by injections of the bodyfluids. If the hormone is transmitted in this manner to a mutant individual which lacks the gene that causes the development of a certain eye pigment, it acquires now the ability to produce the eye color of the dominant race. Such genes thus seem to exert their effects on the recipient tissues by means of hormone-like substances to which certain tissues have a specific affinity. These gene-hormones are not species-specific; they may be effective even in different orders of animals. Ephestia as well as Habrobracon hormones are effective in Drosophila, and conversely, Drosophila hormones exert typical effects in Habrobracon pupae. It is, in all these cases, the wild dark-eyed type which possesses a hormone which is lacking in the mutant form. As to the chemical constitution of such hormones, they seem to be neither protein nor lipoid; they, as well as the organizers, apparently lack organismal differentials.

We see, then, that the organizers, on which the organ formation in the embryo depends to a large extent, and the substances, by means of which the genes produce their effects during embryonal or larval life, are both hormone-like and do not possess the organismal differentials; whereas the substances from which they are derived, the cytoplasm of embryonal tissues and organs and the genes of the chromosomes, have a complex structure and do possess organismal differentials or their precursors. Likewise, the substratum on which they act are bearers of organismal differentials or their precursors. The cytoplasm is the more specific material which has the potentiality to develop and differentiate within certain limits under the influence of these hormone-like inductor substances. The latter induce the development of organ systems in an orderly fashion, in accordance with the organismal differentials of the species and the individual in which they act. Both the precursors of the organizers and the organismal differentials are presumably present in the fertilized ovum. In the course of embryonal life the organ precursors and the

organizers which they contain develop step by step; they become distinct for each organ, until in the end the complete set of organs and organ differentials has fully developed. At the same time, also, the precursor substances of the organismal differentials develop and differentiate into finer differentials, until in the end the structures characteristic of the individuality have fully formed in the substratum. It may be assumed that the coarser organ differentials, organizers and organismal differentials develop first and that only at later periods of ontogenesis the finer chemical structures differentiate in the case of both the organ and organismal differentials. While these two sets of differentials have thus certain important characteristics in common, they differ in their chemical constitution as well as in their distribution. Whereas the organ differentials and their precursors differ in every organ and tissue, the organismal differentials are the same in all parts of an organism. We may perhaps tentatively assume that on a common chemical basis, which is the bearer of the organismal differentials, there are superimposed in various places chemical structures which correspond to the various organ differentials. While the general design of the latter is similar in nearly related organisms, differences develop corresponding to the distance in relationship between the organismal differentials. The finest, the least noticeable differences are found between the organs and tissues of nearly related individuals. Yet, the wider pattern of the embryonal development of the organs and organ differential substances, which takes place by means of self-differentiation and with the aid of organizers, is similar throughout the whole animal series; this applies especially to the coarser, more basic organ and tissue structures, while with progressing ontogenetic development a greater differentiation sets in in the development of organs. These developmental similarities are maintained, notwithstanding the differences which exist as to the precursor substances characterizing the germ cells of the various classes, species and individuals. The organs and their differentials undergo graded changes during embryonal life and they are readily accessible to modification within a certain range, under the influence of alterations in the inner or outer environment. The organismal differentials, on the other hand, although they also differentiate in the course of embryonal development, are, as far as is known, much more stable and much less readily accessible to environmental influences; however, during this period the character of the organismal differentials limits also the variability of the organs which may occur. Differences in organismal differentials which the organizer tissue and the recipient tissue may possess do not preclude the effective action of organizers, but the tissue and organ differentials can develop only within the range prescribed by the nature of the organismal differentials of the recipient tissue.

We have seen that in the adult mammalian organism a tissue equilibrium is established, which is strictly autogenous; the integrity of tissue boundaries, the normal interaction of tissues, depend upon the presence of the same autogenous differential in all the adjoining tissues. On this autogenous character depends the maintenance of the normal tissue equilibrium and the normal function of tissues. There is a good deal of evidence that in the adult mam-

malian organism, also, some special substances are given off by tissues which influence the state of adjoining tissues. They may be contact substances, comparable in certain respects to the organizers of embryonal tissues. Thus, the egg in the ovary may stimulate the growth of the surrounding follicular granulosa and the state of the parenchyma may change the condition of the surrounding stroma; but also, the blood vessels and their permeability may affect the stroma in which they are embedded, and by way of the stroma they may affect even the parenchyma. Local defects may alter the tissue equilibrium, inducing tissue growth, and even without such defects neighboring pigmented epidermis may, under certain conditions, invade unpigmented epidermis. In a similar maner the squamous epithelium of the cervix, which develops under the influence of hormones, may act towards the neighboring cylindrical epithelium of the uterus. These exists, in all probability, other local mechanisms which maintain the tissue equilibrium in addition to the action of hormones originating in distant places. We may then conclude that the normal tissue equilibrium depends (1) upon the action of autogenous differentials, which all tissues possess, and (2) upon a variety of other effects, among which the action of some special hormone-like contact substances as well as typical hormones play a prominent role. There is thus a certain correspondence between the factors which determine the interaction of embryonal tissues and those which determine the autogenous equilibrium of the adult higher organisms.

Chapter 6

Regeneration, Transplantation, and the Autogenous Tissue Equilibrium

In earlier periods of the experimental study of transplantation a discussion arose between two French biologists, Yves Delage and Giard, as to the relation which exists between transplantation and regeneration. Yves Delage maintained that there is an antagonism between these two processes. He based this conclusion on the very great regenerative potency in lower organisms, such as planarians and lumbricidae, which renders transplantation difficult, because the new tissue developing in or near the surface, which separates host and transplant, tends to push off the transplant. Plants, on the other hand, in which the tendency to regeneration is very slight, are very suitable for grafting. However, according to Giard, such an antagonism does not exist. He cited the fact that in tunicates, sponges and corals, where the regenerative power is great, transplantation can readily be accomplished.

In previous chapters we have mentioned the importance of regenerative processes in the fate of transplants; we shall now consider these facts in a connected way, because they have an important bearing on the establishment of the autogenous equilibrium in higher organisms, which holds together the various organs and tissues, as well as different parts, in the same organ or tissue, and unites them into one individual. This equilibrium is autogenous in higher organisms, because adjoining tissues need to possess the same individuality differential. The proof of the existence of such an equilibrium is based largely on the absence of regenerative growth phenomena whenever adjoining autogenous tissues or constituents of the same tissue balance one another in such a way that there is a relative state of rest and a lack of interference with the neighboring tissues. To such a state of formative equilibrium there must correspond a similar equilibrized state of metabolic and functional interactions of tissues; whenever a replacement of the autogenous tissue constituents by homoiogenous constituents alters this equilibrium, regenerative movements and growth tend to take place, and thus antagonisms between adjoining tissues may become manifest; these changes may be taken as an indication that an autogenous equilibrium has existed before the disturbances became manifest. As the following discussion will show, in certain respects there does exist an antagonism between the regenerative activity of the host and the successful outcome of transplantation. There are conditions in which the tendency of the host to regenerate may be responsible for the casting off or the resorption of the transplant; but, on the other hand, there are also conditions in which the transplant may prevent regenerative processes in the host; this it may do if, owing to the nature of the organismal and organ differentials of host and transplant, the contact mechanisms at the point of junction between the

partners are adequate and exert a mutually balancing effect. As stated previously, there is reason for assuming that the normal contact mechanisms depend at least partly on the interaction of adequate contact substances.

The conditions prevailing at the point of junction may influence the occurrence or non-occurrence of regeneration in one of three ways: (1) The presence of adequate contact mechanisms or contact substances may prevent regeneration directly by insuring a relative state of rest; conversely, the absence of such mechanisms or substances may directly cause regenerative processes to set in; (2) the absence of adequate contact mechanisms may lead primarily to the loosening of the connection between transplant and host, and this may be followed by regeneration. In both of these cases we have presumably to deal with specific actions of a chemical nature; (3) the approximation of the surfaces of contact may directly inhibit regeneration in a simple mechanical way by exerting pressure. In addition, we have to consider the growth momentum of both host tissue and transplant; the greater the growth momentum, the greater must be the forces that tend to repress regeneration, other conditions being equal.

While actual experience has proven the mutual antagonism between regenerative activity and successful transplantation, other factors tend to make regenerative processes favorable to transplantation. Thus a slight degree of regenerative activity in many instances is needed for and makes possible the joining together of host and transplant. There may exist, besides, an indirect relation between the degree of transplantability and the degree of regenerative activity which host and transplant exhibit; it depends upon the frequent association of great regenerative power of organisms and their constituent parts, with a primitive, less complex constitution and a correspondingly lower degree of sensitiveness to differences in organismal differentials. There is noticeable, therefore, particularly in phylogenetically and ontogenetically more primitive organisms, a greater mutual adaptability between transplant and host, and a greater ability of the transplant to withstand the injuries connected with the process of grafting, especially during the first critical period following transplantation when the nourishment of the grafts may as yet be inadequate. But where the opposite conditions prevail, where there is a lack of regenerative ability associated with a great sensitiveness of the tissues to injuries, transplantation may be impossible, as, for example, in the case of the adult mammalian ganglia cells of the central nervous system.

It was presumably the difference in point of view between Yves Delage and Giard which, more recently, suggested to Weiss the analysis of the factors on which the antagonism between regeneration and transplantation depends. In Salamander larvae, amputation of an extremity is followed by regeneration of a new extremity; but if, according to Weiss, another extremity of such a larva is transplanted onto the wound, regeneration is completely prevented, provided the new extremity fits the defect anatomically as well as functionally; however, if the covering of the wound by the surface of the transplant is incomplete, wound healing may take place at first, but then regeneration may set in, and even if it is rudimentary or retarded, the transplant is cast off.

These observations agree with those of Morgan, who previously noted that if, in tadpoles, a tail is cut off and the cut-off tail of another larva is grafted onto the wound, regeneration does not occur on the cut surfaces, althougb both the stump and the grafted tail have the power to regenerate.

Similar results were obtained in anuran amphibia by Grāper. Transplantation of extremity buds on stumps of limbs succeeded, but regeneration was prevented thereby only if the orientation of the cut surfaces of host and transplant to each other was correct. If the two surfaces were not adequate, the transplant either changed in such a way that it became secondarily adjusted to the host and was transformed into the right kind of extremity, or, if this did not take place, there was a regeneration of the original limb, notwithstanding the presence of the graft. Of special interest is the fact that in case of a disharmonious character of the cut surfaces a regenerative growth occurred, which did not need to be restricted to the cut surfaces but which took place even at some distance in the transplanted limb. We have already referred to similar results when we discussed regeneration in primitive adult invertebrates, where likewise an outgrowth may take place at some distance from the place of union of the two pieces, a contact effect apparently having been propagated from the directly affected area to nearby parts. Therefore, according to Grāper and Weiss, a satisfactory axial orientation between transplant and host is essential if regeneration is to be suppressed. An arm can inhibit the regeneration of a posterior extremity, provided the axes in host and transplant have an analogous orientation. If the transplantation occurred not directly at the point where a part of the limb had been cut off, but at some distance from it, in the direction towards the head in the branchial region, the tendency to regeneration was greater, but in principle the same competitive struggle took place between the prospective or early regenerate and the transplant, and in certain cases both pieces, regenerate and transplant, coalesced. The transplant, even if it did not succeed in suppressing the regeneration, was able in some instances to make it less perfect.

In many other experiments, also, especially those of Harrison, success in the grafting of extremities in amphibian larvae depended largely upon the fulfillment of the condition that the transplant satisfy the tendency of the host to form a certain type of extremity; unless the transplant conformed to this condition, the reaction of the host tissue was unfavorable to a permanent union. In these cases we have, it seems, to deal with specific interactions between host and transplant at the point of contact. But homoiogenous tissue of a different kind, such as transplanted living skin, may also exert an inhibiting effect on the regeneration of extremities. Thus Harrison and Detwiler found in embryos of Amblystoma that the regeneration of limbs which had been excised, can, to some extent, be inhibited if the wound is covered with homoiogenous skin, and it can be entirely prevented if the wound and the size of the skin subsequently grafted onto the wound are very extensive.

However, there are several investigations which make it very probable that in addition to these specific contact actions, also purely mechanical, nonspecific factors may play a part in preventing regeneration. Thus Schaxel

observed that the covering of a wound in Siredon pisciformis not only with transplanted living skin, which heals on rapidly, but also with dead material, may prevent regeneration. In this case purely mechanical factors are probably responsible for the result and we might even conclude that if the organismal or organ differentials are active after transplantation of extremities, their effect is only an indirect one, permitting the graft to remain in perfect apposition to the wound and thus to exert the needed mechanical pressure; but if the differentials are not compatible with each other and the right contact substances do not interact in the area of the wound, then the transplant is not able to exert the required mechanical pressure on the wound surface and regeneration takes place. But, there is reason for assuming that the type of inhibition of the regenerative process which occurred in Schaxel's experiment is different from that caused by the transplantation of an extremity bud. In the former case regeneration was not actually prevented; it began to take place and then the pressure of the scar-tissue apparently did not allow the regenerating extremity to break through. Therefore, in this instance the regenerative processes were presumably merely inhibited and made abnormal by the mechanical pressure of the overlying skin. Perhaps the inhibition of the development of transplanted buds of extremities was also a pressure effect of the overlying skin, although here the homoiogenous nature of the transplanted skin may also have played a role. On the other hand, if two well-fitting surfaces of extremities or tails are joined together, even the beginning of regeneration can be obviated. In this case we have probably to deal with specific contact effects rather than with non-specific mechanical pressure.

In accordance with this interpretation, and somewhat different from the conclusions suggested by the experiments of Schaxel, are the results obtained by Godlewski, who noted that only living tissue, especially skin with the underlying cutis, was able to prevent regeneration of a tail in axolotl; furthermore, only auto- and homoiotransplants, or transplants belonging to different races but to the same species, were effective. Thus, according to Godlewski, skin of the white axolotl grafted onto wounds in the black axolotl prevented regeneration of the tail in the latter, which would otherwise have followed an amputation. Godlewski assumes that this result is due to the specific effect of the cutis, which remains alive after transplantation and which prevents the epidermis from growing down into the underlying coagulum and initiating the regenerative process. As usual, under similar conditions the inhibition of regeneration is complete only if the wound has been covered in an exact manner. If certain small areas have been left uncovered, finger-like, thin, proliferative buds may grow out.

However, there is considerable difference in the conclusions of various investigators as to the manner in which the regeneration of the extremity takes place. We may cite the more recent experiments of Harrison, who believes that the extremity is produced by the mesenchyme of the extremity bud and not by the ectoderm. Still, the ectoderm may exert some influence on the formation of the limb and different types of ectoderm may vary in the effects

which they produce. While ectoderm taken from the area covering the developing extremities may favor the regenerative growth of embryonal buds, or at least does not inhibit it, ectoderm taken from the head region does inhibit it, but only if this ectoderm has reached a certain stage of development. Similarly, Mangold observed that the epidermis of Axolotl, which does not possess the ability to produce a balancer, may exert an inhibiting effect on skin which otherwise would be able to produce this organ. We would have, then, in this case, to deal with specific effects of the transplanted epidermis on the regenerative process and not with non-specific pressure effects; but while these relations between epidermis and underlying cutis are specific and not purely mechanical in their action, they are specific in a particular way and not exactly identical with the effects observed by Weiss and others. There are involved, here, tissue equilibria of a special nature. According to the observations of Weiss, a transplant inhibits even the onset of regeneration if the two surfaces joining transplant and host are mutually perfectly adequate. Under these circumstances a very rapid union between the two pieces takes place. We may assume that the transplant brings about the same condition at the point of junction which would prevent regenerative growth processes in this area in the normal intact organism; in the latter, the normal neighboring tissue exerts presumably the same kind of inhibiting contact effects as does the grafted, strange tissue under experimental conditions. Inasmuch as in many of these experiments there are successful homoiotransplantations, we may furthermore conclude that even homoiogenous differentials make possible these normal interactions of equilibrating contact mechanisms in amphibia, and also that a very brief interruption of the contact action, such as occurs during the excision of a piece of tissue and the grafting of another piece in its place, is not sufficient to initiate growth processes. But if these contact actions are not completely adequate, graded differences in incompatibility may exist in different cases between transplant and host and then it is possible for the regenerative outgrowth of the host tissue to take place even at a time when the union with the transplant has become already so firm that this outgrowth is unable to induce the casting off of the transplant; instead, a struggle may develop between the two tissues and the transplant may be pushed sidewise by the regenerating host tissue, so that in the end it forms an appendage to the regenerated extremity and a double formation is produced. In this case the mutual antagonism between host and transplant manifests itself in an inhibition of growth of the transplant; but the more subtle mechanisms of attack by means of specialized cells of the host, which we can observe in mammalian transplantation, are, as yet, apparently lacking in these more primitive organisms.

Similarly in the experiments of Milojevich, who used Triton extremities directly after metamorphosis, the surface of an extremity was partly, but not entirely, inhibited from growing out by grafting onto it the regenerative bud of another Triton limb. If the latter was at such an early stage of development that the tissue differentials had not yet fully formed, then the outgrowing part of the remnant of the host bud and the grafted bud united to form one

extremity, but at the sides where the grafted limb did not fully cover the remnant, new extremities grew out. In this instance, therefore, the inhibition exerted by the graft was strictly limited to the place of contact. If, instead of grafting another bud onto the exposed surface of the regenerative bud, it was completely covered with a piece of skin or with a layer of muscle and skin, the regeneration was entirely prevented. Possibly here mechanical factors also played a role, as they apparently did in the experiments of Schaxel.

Another interesting example of the antagonistic action between transplant and host, and the latter's tendency to grow or regenerate, is the inhibiting effect shown, in various degrees, by the morphogenic gill field on the development of transplanted limbs. In the presumptive gill region ectoderm and mesoderm have the tendency to produce gill structures, a tendency which is graded in intensity in different areas (Ekman, Detwiler); this inhibiting effect is evidently of a specific nature and it leads to a struggle between the transplant and the host tissues, which mutually antagonize each other in the realization of their morphogenic tendencies. These effects consist presumably in contact actions. Very fine differentiations which take place during embryonal development in this area are made manifest by means of transplantation, and they determine the character of the contact actions. Thus, in general, the nearer the ectoderm used for transplantation is situated to the gill region in the donor, the more it is forced to conform to the influences exerted by the underlying tissues in this area, which tend to convert the transplant into gill structures and at the same time to suppress limb formation.

The specificity of the factors which are active in the inhibition of regeneration is, perhaps, most convincingly demonstrated in some experiments of Harrison, which concern the production of heteromorphic tails in larvae of Rana. Two anterior parts of these larvae were united, each with the aboral pole of the other. If a piece was cut off from one of the combined anterior parts a tail regenerated, in which the medulla of the head part, which had been left intact—the new host—and that of the second partner—the graft—and its regenerate were united, but in which the chordae were not united. Under these conditions the free end of the chorda of the dominating host stimulated regeneration of an additional tail, which possessed chorda but in which the medulla was lacking. Evidently the surface of the medulla in the graft, which fitted the surface of the medulla in the host and regenerate, prevented a new regenerative outgrowth of the medulla of the host into the additional tail. On the other hand, the surface of the chorda, not being inhibited by contact with a suitable surface of chorda tissue, regenerated and gave rise to the newformation of a tail. In this case, also, the inhibition must have been of a specific character; medulla inhibited medulla, but the chorda, not being specifically inhibited by an adequate surface of chorda, grew out and gave rise to regeneration. Here we can therefore exclude simple mechanical factors as inhibitors of regeneration.

Whether there will be compatibility or lack of compatibility between host and transplant depends also upon the degree of self-differentiation which has been reached in the development of both host and transplanted tissues. As

long as the material entering into these reactions is still plastic, adaptable, and not yet definitely fixed and differentiated, especially in the transplant, there is less likelihood that incompatibilities will develop, than at later stages when differentiation into the more rigid structures has already occurred.

We find, therefore, very complex interactions between transplant and adjoining host tissue, and the effects exerted by neighboring tissues upon each other depend not only on the kind of tissues which are brought into contact with each other, but also on the stage of development and differentiation of these interacting tissues. Thus the inhibiting action of a transplant on the regeneration of an extremity is effective only in the first phase of the process of regeneration; it is ineffective if the transplantation is carried out at a later stage, when regeneration is already under way. On the other hand, if in some manner, as for instance through a purely mechanical factor, we prevent the regeneration from being initiated, all subsequent outgrowth has, by these means, been made impossible. Perhaps the ability to regenerate depends upon the presence and activity of a sensitizing substance, which may be lost or neutralized after a definite time has elapsed. This would represent a condition analogous to that observed in mammalian organisms, where a placentoma can develop only at the stage of the sexual cycle when the sensitizing substance given off by the corpus luteum has become active. The effect of certain contact actions would then perhaps consist in a neutralization of the influence of sensitizing and stimulating substances.

It follows from our previous discussions that these contact mechanisms between adjoining tissues may consist in the giving-off of various specific substances corresponding to organizers, to sensitizing, or, under some conditions, also to inhibiting substances in the place of union. In addition, the physical-chemical structure of the cut surfaces of transplant and host may be of importance, in accordance with Gräper's comparison of these surfaces with electro-magnetic fields.

The importance of contact effects in determining the fate of tissues is indicated also in some experiments of Schaxel with transplantation of extremities in Axolotl. If buds at very early stages of regeneration are transplanted into a further developed body wall, the transplant is not able to form an extremity through self-differentiation; it is prevented from doing so by the organizer action of the strange surrounding host. Instead, the transplants may form irregular masses, which later disappear; but further differentiated regenerative buds transplanted under the same conditions are able to form extremities. However, if an early regenerating bud is transplanted together with the surrounding skin, then it may differentiate into the typical extremity; apparently its own skin can supply the needed kind of contact action, which allows it to differentiate normally and to maintain itself after transplantation.

We can understand the way in which neighboring tissues exert contact actions upon each other, presumably through the giving-off of certain substances, if we consider what happens at certain stages of metamorphosis. In anuran amphibia the gills at definite periods of metamorphosis secrete a substance which dissolves the overlying skin. Also, transplanted gills exercise

this function, but later on they lose it. In these instances there may be active the secretion of an acid or of a proteolytic enzyme possessing the power of dissolving the skin and serving as a contact substance. In a corresponding manner, Helff has shown that it is due to absorption processes taking place in the gills, which must be in direct contact with the integument, that histolysis in the overlying integument is initiated. The histolytic influence of the atrophying gills increases at first as metamorphosis proceeds, reaching a maximum just prior to the release of the forelimb; and then gradually it subsides as the gills undergo the final stage of atrophy.

As stated previously, we think it justifiable to transfer these conceptions, derived from what has been observed under experimental conditions, especially those prevailing after transplantation, to the equilibrium, which exists normally in an organism, between adjoining tissues; here contact substances, in addition to hormones, presumably determine the tissue equilibrium, and disturbances of this equilibrium may lead to extensive regenerative processes in phylogenetically or ontogenetically more primitive organisms, and to simple wound healing in the more differentiated organisms. Among these contact substances the organismal as well as organ differentials may play a part, the organismal differentials gaining in importance with increasing phylogenetic and ontogenetic development.

Certain kinds of transplantation in the more primitive classes of invertebrates similarly contribute to the understanding of the significance and origin of regenerative processes at or near the point of junction of graft and host and to the interpretation of the factors that maintain the tissue equilibrium within the same individual. We have seen that organismal differentials are of importance in this process, as are also correct axis orientation and polar direction of joined parts. This is true especially in the case of the more primitive invertebrates as well as of plants. If the cut surfaces do not fit each other completely, a regenerative outgrowth may take place from an uncovered point. Moreover, in vertebrates as well as in invertebrates, regenerative processes may proceed not only directly from the free surfaces of injured organisms, or from surfaces exposed after incompatible pieces have separated, but also from totipotent cells which migrate to the exposed surfaces. Such observations have been made, for instance, in amphibia by Hellmich, and by Spek and others in the ascidian Clavelina. In the latter case, under various conditions leading to budding, certain totipotent cells are attracted from the deeper tissues to that point of the body where the growth processes are to take place. It may be assumed here, too, that certain substances rather than purely mechanical factors direct the movement of these cells. It seems that the separation of the transplanted parts may in some cases constitute the primary process, which subsequently is followed by regeneration; but in other cases, as we have previously pointed out, it is very probable that incompatibilities between the joined pieces lead to regenerative processes, which are thus primary, and that these are followed only secondarily by a separation of the parts.

Cell equilibria which depend upon contact influences exerted by adjoining cells upon each other, determine whether one or more embryos shall develop

from the blastomeres; this is a problem which we have already discussed in a previous chapter. Developmental processes which might lead to the formation of two embryos are prevented if the surfaces of the blastomeres, either derived from the same or from different eggs, are oriented to each other in the right direction and if the organismal differentials of the joined parts are mutually compatible. Under these conditions adjoining cells, even if they were obtained from different organisms, may restrain each other from carrying out movements and from undergoing cell divisions, such as would give rise to the formation of a whole organism from one of the partners; instead, the blastomeres may coordinate the activities of the neighboring cells with their own.

However, if the organismal differentials of the partners are unsuitable, or if the axes of the adjoining segments do not fit each other, then the neighboring segmented cells no longer exert this regulating effect. When unsuitable heterodifferentials cause the duplication of organisms, the two partners may still remain united in a mechanical sense; but sometimes a complete separation occurs. Conversely, in the normally segmented ovum each blastomere may develop into a separate individual if the surfaces through which the blastomeres are joined are altered, or if the substances lying at the surfaces of the cells are made to move. The same conditions in the surrounding medium which prevent the spontaneous separation of joined together blastomeres and the subsequent initiation of abnormal growth processes, may also bring about the union of two organisms into one. In regeneration in both adult and in embryonal tissues the character of the organismal differentials, the nature, and in particular, in certain cases, also the orientation of the parts of cells or tissues adjoining each other, determine whether or not movements of cells, as well as cell multiplications, shall be initiated, which may lead to the formation of separate organisms; in the case of the ovum, movements of special substances also play a role in this regard.

In general, transplantation of suitable tissues onto remnants of embryonal tissues prevents regeneration of the host embryonal tissue, and conversely, the latter may prevent such growth in the transplant; but if various incompatibilities exist, these act as stimuli which may cause an outgrowth from the host or a duplication of the transplant. Such incompatibility may consist in differences in organismal differentials or in the contact of otherwise unsuitable tissues; even the turning around of a longitudinal axis of one of two, ordinarily suitable, tissues may bring into contact unsuitable tissues. But, an embryonal bud does not tend to reduplication if the strangeness of the soil onto which it is transplanted exceeds a certain limit of unfavorableness. Thus, if limb buds are transplanted to the head or medulla of larvae of salamander, conditions which favor duplication are lacking.

We find, therefore, that very early embryonal buds of amphibia behave in a similar manner to adult organisms of very primitive classes of animals; also, that very young embryonal material and early regenerative stages in adult primitive animals behave very much alike. In all these cases, we have to deal with plastic material, where a certain degree of unsuitability between

the part of an organism and the environment may lead to growth processes tending to the reduplication of the tissues. In such a finely equilibrated system the normally present factors must cooperate to keep an organ, or a part of an organism, at rest. Furthermore, a normal, non-transplanted part of an organism which still tends to grow, may be induced by the presence of an otherwise indifferent foreign body to produce an additional extremity, provided the necessary material for such an outgrowth is present. Or in very primitive organisms, such as planarians, disturbance of the equilibrium by mechanical means may lead to fargoing transformations in the individual, and in coelenterates changes in the oxygen content in the surrounding medium, or perhaps also diffusion of growth inhibiting substances out of the animal may be followed by the formation of multiple growth centers. It may be assumed that the contact with suitable tissues maintains an equilibrium in which all parts of the organism are correlated in such a way that abnormal growth processes are excluded; distance substances also play a role in maintaining this equilibrium. If these normal contact actions are interfered with, outgrowths, which may lead to reduplication in some cases, take place in very plastic material, while simple wound healing follows in higher, more differentiated organisms. In all these instances the alteration in the environmental condition represents the first link which sets in motion a chain of events leading to the abnormal growth. It is of great interest to note the apparent similarity in the initial factors, as well as in the subsequent links of the reaction chains, which play a role in embryonal development, in budding, in the regenerative newformation of organisms, and in the more simple wound healing as we know it in higher organisms.

Whether an outgrowth occurs from a tissue surface which is not adequately covered by other tissue, depends also on the growth momentum inherent in the substratum. The greater this momentum is, the greater the restraining action of the transplant must be to become effective. The growth momentum is highest in the more primitive organisms. Here, too, transplantation of tissues succeeds better and differences in organismal differentials between host and transplant play a less important part than in higher organisms. In the latter the transplants have to overcome greater difficulties in holding their own, but they have not to overcome as great a growth momentum in the host as do the transplants in the more primitive organisms. Tissue transformers in the form of organizers are lacking here, where the substratum has lost its plasticity.

As to the character of the contact mechanisms, we have, as stated above, presumably to deal with substances or chemical groups transmitted from one surface to an adjoining one; conditions here seem to be analogous to those observed in the case of the organizers, where effects exerted by chemical substances are involved. Grafting experiments in embryonal and very primitive adult organisms confirm and extend, therefore, our conceptions as to the part which contact mechanisms play in higher and fully developed organisms. There takes place a gradual transformation of the embryonal system of regulation into the system of regulation of the higher adult organism, which, because of the prominence of the organismal differentials, becomes an autogenous regulating system. This regulating system functions in higher organ-

isms through the tissues, which are the carriers of finely graded organismal differentials.

In the highest organisms, the adult mammal, the same factors which are active in the lower organisms play a role in the maintenance of the equilibrium which makes possible the existence of an individual. But in contradistinction to the findings in more primitive organisms, this equilibrium is an autogenous one. The various tissues composing the individual must have the same individuality differential, otherwise disturbances take place. In addition, also mechanical factors, like cuts, the presence of foreign bodies, may lead to disequilibrations in these organisms, which are, however, usually readily repaired. Only under certain conditions of sensitization may mechanical factors lead to furthergoing growth processes, such as the formation of placentomata. But even without the action of mechanical factors the autogenous equilibrium may be disturbed if growth stimuli act on adjoining tissues of a different kind; thus, changes connected with transplantation of pigmented skin into defects in white skin in the guinea pig may give to the pigmented skin, or some of its constituents, a growth momentum which causes it to invade the adjoining white epidermis. Similarly, if in the vagina-cervix-uterus sex tract a marked and long-continued stimulation of the surface epithelium is produced by the injection of estrogen, the growth momentum of the epithelium of the cervix, which has the power to produce squamous epithelium, is increased more than that of the cylindrical epithelium of the uterus, and in consequence of this disequilibrium the squamous epithelium may invade and replace the cylindrical epithelium over long distances. The equilibrium in the normal individual depends, therefore, also upon the maintenance of the mutual normal growth momentum of adjoining tissues. A long-continued disturbance of this equilibrium by a variety of factors may ultimately lead to the initiation of localized cancerous growth.

In general, we may then conclude that a finely equilibrated state exists between neighboring tissues, the disturbance of which may lead to growth processes which, in some cases, succeed in restoring the same, or, in other cases, a new stable equilibrium. Transplantation prevents regeneration when it supplies the missing regulatory factors, which in the higher organisms are of an autogenous character; but in principle, conditions are the same in this respect in the furthest differentiated adult organisms as in the more primitive and embryonal ones. In the latter, regeneration can be prevented by tissues which differ within a certain range in their organismal differentials, and which also may differ in their tissue differentials. We can here distinguish (1) a specific inhibition exerted by tissues of the same kind, such as, for instance, medulla restraining adjoining medulla, or chorda restraining chorda, in their respective regenerative tendencies (isoregulation), and (2) an inhibition by tissues of another kind, such as gill tissues inhibiting leg growth, or skin preventing the growth of a tail or limb (alloregulation). It is necessary, besides, that transplant and host, or adjoining tissues in general, should be in close contact if the specific interactions between neighboring tissues are to become effective; otherwise these interactions are interfered with and growth and

movements may set in. Hence inadequate mechanical factors may be the primary link which leads to these reaction chains.

The results of these various sets of experiments so far discussed harmonize with each other and also with the conception of the role that autogenous morphogenic contact substances play in determining the tissue equilibrium. Again, in this instance the reactions which take place if incompatible organismal differentials are joined together, are due not merely to mechanical factors, although mechanical factors are involved too in the maintenance of the autogenous tissue equilibrium, but to the interplay of chemical contact factors of a more specific character which reside in the tissues. The effect of unsuitable contact substances may be transmitted to neighboring areas.

The higher developed the organism, the finer and more differentiated are the organismal differentials which keep the various parts of the body in equilibrium; this applies to the relation between neighboring parts of the same type of tissue, as well as to the relation between neighboring tissues which differ in type. In the more primitive organisms the individuality or species differentials do not yet possess the same fineness as in higher organisms; at least the more delicate reactions, which would allow their manifestation, are lacking. Correspondingly, in these organisms embryonal or regenerative organ and tissue formation is still possible and here, too, organismal transplantations can be made.

We now have analyzed two sets of facts in connection with the development of the more primitive into the higher organisms. In the first place we have noted the importance of organizer actions, which are very potent in early embryonal stages, and their replacement with advancing embryonal life by very complex systems of contact substances, functioning between adjoining parts of tissues and organs. Involved in this process, also, is the action of distance substances or hormones, and, moreover, a step-by-step diminution in growth potentialities and growth momenta, as well as in tolerance to strange organismal differentials, as tissues and organisms progress from primitive to more differentiated types.

Furthermore, through transplantation experiments we have arrived at the recognition of the relatively rigid character of the adult higher organisms. Reactions of growth and differentiation are here very much diminished, except in cancer growth, where the growth momentum of tissues may be very great. Not only are the reactions against strange organismal differentials very strong, but there exist, besides, some reactions against strange tissue differentials. While in the more primitive organisms the organismal differentials play a relatively less significant role and the interactions of tissues and organs and the transformations which they undergo are very prominent, in the higher organisms, concurrently with the diminution in potentialities of growth and differentiation, and in morphogenic effects in tissues and organs, the reactions against organismal differentials become very pronounced. There are found in the higher organisms a marked fixity and strict regulation of tissues and organs, which latter is maintained by the interaction of contact substances. Thus an autogenous tissue equilibrium, which makes possible the existence of integrated individuals, is established.

Part III The Significance of Organismal Differentials in the Interaction Between Single Cells

Chapter I

The Role of Organismal Differentials in the Union of Free-living Cells

WE HAVE SO FAR considered the significance of organismal differentials in the grafting of pieces of tissues or organs, or of whole organs, to embryonal or adult organisms, as well as in the union of larger parts of primitive organisms and in parabiosis. As a further step in the analysis of individuality, we shall now study the role which genetic relationship and the organismal differentials play in the joining together of parts of cells or of whole cells, which latter may function as independent, free-living organisms. In these experiments we have not to deal with transplantations in the usual restricted meaning of the term, but with related processes. The methods used and the problems considered in this part are similar to those studied in the previous parts. We should naturally have to include in these chapters also experiments in which unsegmented eggs or ova in early stages of segmentation were joined together; however, these have already been discussed in earlier chapters, in which in experiments with the eggs of Ascaris distinct effects of the organismal differentials or their percursors were noted, and this was true also in the experiments of Mangold on the combinations of eggs in amphibia. We have also reported already on investigations in which early embryos or parts of embryos were united.

In this chapter analogous phenomena in certain protozoa and unicellular plants will be analyzed.

1. *The union of free-living protozoa or of parts of protozoa.* As early as 1863, Max Schultze observed that pseudopods from different individual protozoa belonging to the same species did not unite when they were brought into close contact with each other; but it was only in 1897 that Jensen noted a difference in the behavior towards each other of protoplasmic particles from the same and from other protozoan individuals. In experiments especially with the polythalamous rhizopod Orbitholites, he observed that two pseudopods from the same individual readily joined each other at the point of contact to form one single organ, and in particular, adjoining small pseudopods could unite into a single larger one by the flowing together of the protoplasm at the points of contact; furthermore, a pseudopod of large size could incorporate a smaller one. In these cases we have to deal with autogenous reactions. On the other hand, if two pseudopods which belonged to two differ-

ent but homoiogenous individuals touched each other, the pseudopods, instead of coalescing, contracted and disintegrated into small balls, which could be taken into the body of the individual from which they were derived. These observations were made primarily on pseudopods which were still connected with the body of the rhizopod. But even pseudopods which had been cut off from the main body behaved in principle in the same manner; they readily coalesced only with the pseudopods derived from the same individual. If, on the other hand, a cut-off pseudopod was first allowed to degenerate and then to come in contact with another individual of the same species, it could be eaten by the latter. This observation suggests that during the process of degeneration the protoplasm loses its individuality differential and becomes converted into inert material that may serve as foodstuff.

If we bring into contact with each other, instead of autogenous or homoiogenous parts, pieces belonging to different species of polythalamous rhizopods, the initial reaction of repulsion, which characterizes homoiogenous contacts, is lacking. Such pseudopods behave to each other in the beginning as they would to foreign material, such as various foodstuffs, with which they first agglutinate and which they then incorporate into their body. But in the case of heterogenous contacts this initial agglutination reaction is followed soon afterwards by a contraction similar to that observed when two protoplasmic particles of homoiogenous origin come into contact, and a secondary separation of the two strange pseudopods takes place. The response to heterogenous protoplasm represents, therefore, a combination of the reactions which take place against a foreign body and of those that occur in contact with homoiogenous protoplasm. But in addition there may be an effect which suggests the action of a toxic substance; for instance, in some cases if a rhizopod touches the pseudopod of a foraminifera belonging to a different species, the rhizopod may be paralyzed and drawn into the body of the latter organism, although it may subsequently be able to free itself again.

It is furthermore of great interest that, to judge from the data available regarding Orbitholites, the reactions of nearly related, syngenesious organisms towards each other may be like those of autogenous parts of a single individual. Thus different individuals may fuse and form a colony. Jensen considered the early age of the individuals which unite as the principal factor underlying this reaction, but it seems probable that the close relationship between the organisms and the great similarity of their individuality differentials are of greater importance than the age. This interpretation is supported by the observation that also in Arcella syngenesious pieces may behave in a similar way to autogenous parts of an organism.

In other organisms, such as Difflugia, somewhat related but less sharply differentiated effects are noted. While here, again, two autogenous fragments of protoplasm may coalesce, homoiogenous particles as a rule react differently towards each other, although occasionally the homoiogenous and autogenous parts behaved alike. In the case of Arcella potyposa, the more recent experiments of Reynotds confirm the earlier observations, according to which autogenous pseudopods which come in contact with each other

readily coalesce; on the other hand, homoiogenous pseudopods which contact each other shatter into small particles or droplets; but this does not apply to the main bodies of these organisms, which are more resistant. Shattering apparently represents a characteristic homoio-reaction and it is lacking if heterogenous individuals come in contact. On the other hand, heterogenous protoplasms do not fuse with each other as readily as autogenous ones. The heterogenous reaction resembles, in certain respects, that noted towards foreign material; however, these heterogenous particles, in contrast to foodstuffs, are not incorporated into the main body of the protozoa.

The dependence of the reactions of individual protozoa on relationship is evident also in the subsequent investigations of Reynolds. He started with a single individual in Arcella, which, in the course of time, underwent fissions, and this process was continued through several generations; a comparison was then made between the behavior of the individuals towards each other in the later and in the earlier generations of such cultures. Reynolds found that although all these individuals were originally derived from a single cell, after some time they began to react towards each other as if they were homoiogenous organisms, and shattering occurred if two such individuals belonging to later generations met. Such a change from an autogenous into a homoiogenous reaction took place after about twenty-two consecutive fissions, even in cells which had been kept under the same environmental conditions, in the same culture fluid. However, if individuals developing through fission of the same protozoon were separated from each other at once and kept in different culture fluids, representing a somewhat different chemical environment, then the homoio-reaction was attained sooner.

But Reynolds was also able to obtain the reverse transformation. For this purpose he proceeded in the following way: after he had changed syngenesious individuals into homoiogenous ones, he succeeded by means of daily exchange of the culture fluid—placing Arcella A into the fluid in which Arcella B had lived—in transforming the homoiogenous reaction back into a syngenesious or an autogenous one. If such individuals were kept together in the same culture dish, the return to the autogenous reaction could be obtained even sooner. It appears then, that we have, under these conditions, not to deal with rigid, mutation-like changes in the protoplasm, but with changes of a more labile nature, which occur in response to environmental factors and that these changes are reversible. This holds good provided the genetic constitutions of the individuals were closely related to each other from the beginning, as is the case if the organisms are derived through fission from a single individual.

Such experiments suggest that into the culture fluid substances diffuse which are characteristic of the individual organism and with which presumably their surface layers become impregnated. These substances would then be responsible for the type of reactions that follow the meeting of two individuals, or at least be one of the factors involved. It must further be assumed that the protoplasm of these organisms is readily modifiable and that in the course of continued fissions a change gradually takes place, leading to a corresponding modification in the character of the substances which they give

ent but homoiogenous individuals touched each other, the pseudopods, instead of coalescing, contracted and disintegrated into small balls, which could be taken into the body of the individual from which they were derived. These observations were made primarily on pseudopods which were still connected with the body of the rhizopod. But even pseudopods which had been cut off from the main body behaved in principle in the same manner; they readily coalesced only with the pseudopods derived from the same individual. If, on the other hand, a cut-off pseudopod was first allowed to degenerate and then to come in contact with another individual of the same species, it could be eaten by the latter. This observation suggests that during the process of degeneration the protoplasm loses its individuality differential and becomes converted into inert material that may serve as foodstuff.

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trorhiza, a foraminifera, E. Schultz found that the pseudopods of two separate individuals can fuse with each other, but it is not certain whether this represented a syngenesio- or a homoio-reaction. Similarly in Radiolaria, Verworn (1892) succeeded in exchanging the nuclei between different, nonrelated individuals of the same species. Such organisms remained apparently normal and were protected by the possession of a nucleus of homoiogenous character against the injurious effects which otherwise would have followed loss of the nucleus.

The extensive studies of Jennings, Sonneborn, and others, make it very probable that genetic factors play a role in the mating reactions in Paramaecium bursaria and aurelia, and similar conditions have been observed in the green algae, Chlamydomonas, and other flagellates, by Moevus. In Paramaecium bursaria, Jennings observed that conjugating pairs can be obtained from mixtures of two appropriate clones, but not from either culture separately. Within a few seconds after mixture the individuals have agglutinated into small groups. If pairs of two agglutinated individuals form, the partners in each pair are derived one from each of the two clones. These mating reactions occur provided certain physiological conditions, such as temperature, light and state of nourishment of the Paramaecium are suitable, and the agglutination takes place if two clones of different reaction types are mixed. But in certain clones, isolated pairs may be observed even between members of the same clone, if the clone is left for a long time in a state of declining nutrition; the latter favors agglutination and this environmental factor may overcome conditions inherent in the constitution of the different individuals. A segregation into two different mating types may occur in some cases at the first division after conjugation; in other cases, all clones descended from the same pairs may represent at first the same reaction type and a segregation may take place only at later fissions. The meeting of two mates is accidental, but an effective agglutination occurs only if the organisms belong to different and suitable mating types. These two individuals remain united for 24 to 30 hours and during this time they exchange half of their chromosomes; however, there is no distinction between males and females in the sense that one family would consist of males and the other family of females. After separation, each parent multiplies by fission. The offspring is at first immature and has not yet acquired the ability to undergo an effective agglutination with an appropriate mate, but in the course of months they become mature. The offspring of two parents that mated are all of the same type, which is usually one to which one of the parents belonged; but in some instances, they may belong to another type. It seems, then, that it is not solely the genetic constitution which determines the mating type.

Likewise, in experiments of Sonneborn, which preceded the ones just mentioned, inheritable differences in mating types were observed in Paramaecium aurelia. Here, in various stocks, collected in different localities, six mating types could be distinguished, namely, types I, II, III, IV, V and VI. Mating occurred only between types I and II, between III and IV, and between V and VI. These three mating groups do not mate with one another;

off. The experiments also suggest that it is the chemical character of the surrounding medium which is responsible for the changes taking place in the constitution of the individual. It is known that protozoa can become adapted to certain toxic substances and to higher temperatures; here likewise, the alterations of the individuals may be reversible. Do we have to deal in these cases with functional, phenotypic changes in these unicellular organisms or in certain parts of them, or do we have to deal with changes in their genetic constitution?

The observations of Reynolds in Arcella bear some resemblance to reactions noted in certain of the higher vertebrates, by means of which the latter are able to distinguish not only between species, but also between individuals or related groups of individuals within the same species; as an instance, we may cite the recognition by dogs of individual scents. In the latter phenomenon true individuality differentials are not involved, but the characteristics used for differentiation between individuals are localized in certain organs and tissues.

However, one important feature these reactions in protozoa have in common with the reactions due to individuality differentials in higher organisms, namely a graded differentiation between different organisms in accordance with their relationship; this similarity may be taken as an indication that also in certain protozoa differences exist in the constitution of individuals as well as of different species. We may then provisionally hold that the reactions which we have studied in this chapter are due to substances analogous to but not identical with organismal differentials, substances in particular analogous to individuality differentials. It may furthermore be assumed that in protozoa, too, a differentiation of cytoplasmic constituents and also of genetic substances has taken place in the course of evolution, which has made the production of such substances and the manifestation of these mechanisms possible. It seems that a finely adjusted constitution of the surface layer of these protozoa has made possible the individuality, race and species reactions, which take place when two individuals or parts of individuals come in contact with each other. However, in addition to contact actions, the organisms seem to exert upon each other also some distance actions. Thus, according to Reynolds, Arcella moves in the direction towards detached autogenous or syngenesious pieces of Arcella, but it is not attracted by fragments of individuals belonging to the species Difflugia. The distance reactions and the substances on which they depend are apparently not so finely graded as the contact reactions. We may perhaps interpret, in this sense, the observation that parts of an Arcella, which in the course of generations has lost an autogenous or syngenesious contact reaction, the latter having been changed into a homoiogenous reaction, may still retain an autogenous reaction towards Arcella if it is not in direct contact with it. While the contact action may depend upon sessile or only slightly diffusible substances, the distance reactions in all probability are mediated by diffusible substances.

But, not in all unicellular organisms have such finely graded reactions, indicating the relationship between individuals, been observed. Thus in As-

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the organisms belonging to these groups differ also in other characteristics and represent, therefore, three physiologically distinct varieties. Certain races belonging to variety 2 produce toxic substances, which may be the same or different in different races. One of these substances is *strongly* toxic for all the races belonging to varieties 1 and 3, and only *weakly* toxic for races belonging to their own variety, namely, 2. This toxic substance affects, markedly, also certain other species of Paramaecium. Similarly, the toxic substance produced by another race of variety 2 exerts marked deleterious effects on all the races of varieties 1 and 3, but is very injurious only for certain races of its own variety, whereas others are more resistant or completely immune. This toxic substance also acts strongly on certain strange species, but not on others. In these cases we have evidently to deal with a reaction type, which we have designated as "specific adaptation," the specific adaptation depending in this instance presumably upon the specific chemical nature of the toxic substances and of certain receptor substances in the different varieties, which insures a decreased toxicity of a substance secreted by Paramaecium aurelia for nearly related organisms. Sonneborn made it probable that Mendelian rules of inheritance are applicable in the transmission of the characteristics determining reaction types to successive generations of the various races. The determining factors were contained in the micronucleus, but exerted their influence by way of the macronucleus and cytoplasm. However, these genetic factors were accessible to environmental conditions, and, in particular, the temperature prevailing at the time when the macronucleus is formed from the micronucleus could influence the proportion of individuals belonging to certain types. After this sensitive period has passed the mating type is inherited by all subsequent macronuclei produced at later fissions without further interference by the temperature. In other organisms studied by Moevus, light is able to suppress the mating reaction and in certain cases the mating types seem to be determined entirely by environmental factors.

We see, then, that in interactions of certain protozoa, comparable to fertilization reactions in higher organisms, factors play a role which tend to prevent fertilization with nearly related organisms and favor fertilization with selected, more distant groups of the same species, and that differences in reactions may have to be attributed to genetic differences in the constitution of clones; there are, furthermore, indications that also in these protozoa changes in the constitution of the genetic substance may take place and thus increase the diversification of various stocks. In contradistinction to these genetic differences between various stocks of Paramaecia just discussed, certain structural abnormalities, which may be found in some individual Paramaecia raised in cultures, do not seem to affect the mating reactions, inasmuch as such abnormal individuals can be made to fuse with normal ones under the same conditions as can other normal individuals.

Is it correct to attribute these reactions between different groups of Paramaecium to mechanisms comparable to those occurring in higher organisms under the influence of organismal differentials? There are some apparent

similarities between the reactions noted in Protozoa and in higher organisms, but there exist also marked differences. The characteristic feature of organismal differentials that they are the same in the various tissues of the same organism and are different in the analogous tissues of different individuals does not apply to unicellular organisms. Furthermore, it is very probable that many genes enter into the constitution of the individuality differentials and of the organismal differentials. In Paramaecia, on the other hand, there are strong indications that the difference in agglutination reactions depends upon single or a few selected genes. It seems then that the reactions between different mating groups of Paramaecia are analogous to the fertilization reactions in higher organisms and this is also implied in the term "mating reactions" given to this condition, or they may be compared to the agglutination reactions between different blood cells belonging to different blood groups in higher organisms.

Reactions similar to those studied in protozoa have also been observed in algae and myxomycetae. In the phycomycetous fungus Achlya, the sexual reaction between male and female mycelia seems to depend upon the action of hormone-like contact or distance substances. Such a substance given off by the female vegetative hyphae induces in the male the formation of antheridial branches and the oogonial initials attract the antheridial branches, causing the delimitation of the antheridia. The antheridial branches on their part act on the female vegetative hyphae and here induce the formation of oogonial initials, and furthermore, the antheridia cause the delimitation of the oogonia through the formation of a basal wall. These reactions take a normal course if the male and female organisms belong to the same species, but if male and female belong to different species of Achlya, the reaction sets in but remains imperfect. It stops either at the time of the differentiation of the antheridia, or the female fails to produce oogonial initials in response to the substances produced by the numerous antheridial branches. This indicates a specific adaptation between these distance substances, which transmit the stimuli from male to female, or vice versa, and the mycelial substratum on which these substances act. If the latter and the substratum are derived from different, though related, species, the reaction will be incomplete.

We may refer here, also, to the very interesting recent investigations of Moevus concerning the motility, chemotaxis and copulation of the gametes of certain green algae. There exist a number of races or species of Chlamydomonas which show inheritable differences in the mode of reactions of their gametes and the conditions which determine these hereditary differences are localized in the chromosomes of the various races. As a result of these genetic differences race specific substances are produced, which direct the motile gametes in the dark. The extract from individuals of each race or species acts most efficiently on the gametes of their own race or species, and more weakly on the gametes of other races. These specific substances are carotinoids; the filtrates contain *transcrocetin sugar esters* which are responsible for these effects. Also, the sugars which combine with crocetin seem to be specific in the different races.

The copulation-determining substances are, or at least act like different combinations of cis- and transcrocetin methyl esters and the proportions of these two esters differ in the different races of Chlamydomonas eugametos and also in certain other species of Chlamydomonas. These proportions are hereditarily fixed for the gametes of these races and species; copulation occurs between those gametes of races and species in which the difference in the proportions of these two esters exceeds a certain threshold value. This same difference in the proportions of the esters determines also the degree of chemotactic action which must precede copulation and which leads to group formation in the various gametes, processes of agglutination being presumably involved in these relations. The interactions between certain cells are thus determined by substances which cause distance as well as contact effects and they are graded in accordance with the genetic relationship of the different races, and the combinations of the effective substances are likewise thus graded.

In the alga Bryopsis, Prowazek (1907) has apparently observed, if we interpret his short description correctly, that when protoplasmic particles, which are surrounded by haptogen membranes, come into close contact with each other, the membrane dissolves and the particles coalesce, in case we have to deal with substances derived from homoiogenous organisms; but if the particles belong to different races or species, such a solution of the membrane and fusion of the protoplasms do not take place.

Similarly, in the formation of plasmodia of myxomycetae, individual myxomycetae or small plasmodia first stick together and then coalesce into one large plasmodium. Occasionally such a coalescence may take place even between a large active plasmodium and a small resting round plasmodium, which had previously been taken into the body of the larger individual (Celakowsky, 1892). However, as Cienkowski (1863) had found previously, only plasmodia or myxamoebae of the same species can coalesce. If heterogenous individuals meet, they may flow around each other but do not unite, even individuals belonging to nearly related species differing in this way from individuals of the same species. Whether only syngenesious or also actual homoiogenous individuals coalesce with each other is not stated by these authors, but it appears probable that all individuals belonging to the same species can thus unite. Nevertheless, there have been observed instances in which even separate parts of the same cell could not join each other; this was the case when haptogen membranes developed on the surfaces of the particles.

The tendency to react adversely to contact with the protoplasm of other individuals of the same species, which has been found in certain rhizopods and which we have discussed already, must have the consequence that such organisms, even when not surrounded by a shell or cuticle, remain separate. But if a syngenesious reaction should become identical or almost identical with an autogenous reaction, then the formation of larger plasmodia or colonies would not be impossible. Conversely, it may be expected that in organisms which tend to form plasmodia or colonies, this sensitiveness to homoiogenous protoplasm is lacking and an antagonistic reaction takes place only if more pronounced differences between the organismal differentials of two individu-

als exist. It would be of interest to compare, from this point of view, the protoplasmic reactions in different colony- and plasmodia-forming organisms with the corresponding reactions in types of organisms which live as isolated individuals.

We have seen that genetic relationship may determine not only the character of contact reactions, but may control also the movements of two organisms which are at some distance from each other. Analogous reactions occur likewise in cells of metazoa. The cytotropic reactions described by Roux (1895) may possibly be of a similar nature. Roux found that cells of morulae or blastulae of Rana, when separated from each other at no greater distance than the diameter of a cell, may send out processes and move towards each other. However, in this case reactions between homoiogenous cells were found to be apparently of the same character as autogenous reactions. Cell movements, which probably depend upon substances active at a distance, seem to play a role also in embryonal development. For instance, in urodele larvae certain mesoderm cells are attracted by and move towards the developing eye vesicle; in this case the organismal differentials have not yet reached a stage of marked specificity, and accordingly a lack of individual specificity is noted in the movements of these embryonal cells towards a transplanted eye vesicle. As we have seen, in higher organisms the organismal differentials regulating the interaction between cells and tissues are more finely graded. In addition to the examples discussed already, we may mention the following observation recorded by A. Fischer. If parts of two homoiogenous chick embryo hearts are combined in vitro, cellular anastomoses between contractile elements of such fragments are produced and synchronous pulsations of the two parts take place; but this reaction does not occur if the embryonal heart fragments, placed in contact with each other under otherwise the same conditions, belong to two different avian species. In a like manner, as we have already pointed out, there is reason for attributing to autogenous morphogenic contact substances the function of maintaining in mammals the normal inter-relation and balance between the different tissues of the same organism, the autogenous tissue equilibrium.

It seems then that reactions occur in unicellular organisms, which in some respects correspond to those which, in higher organisms, we attribute to organismal and especially also to individuality differentials. But as we have seen, the same criteria as to organismal differentials which we applied in higher organisms cannot, in a strict sense, be used in protozoa and unicellular plants, because by definition the organismal differentials, and in particular the individuality differentials, are substances present in all, or almost all of the cells and tissues of a given individual and differentiate this individual from all other individuals. It is clear that in a unicellular organism such a definition cannot apply. Still, we may at least conclude that certain protozoa and flagellated gametes of algae possess structures and substances which function in a somewhat similar manner to the organismal differentials of higher organisms, inasmuch as they determine in a graded manner the reactions of these cells to other cells in accordance with the genetic relationships. Also, the male

and female mycelia of certain fungi may behave in an analogous manner. However, we have also pointed out some important differences between the mechanisms underlying apparently analogous reactions in these primitive organisms and in vertebrates. It is possible that not all the reactions in these primitive organisms are of the same kind, and in some of those which we have described the probability that individuality differentials are involved is greater than in others.

We may find even in some of these unicellular, apparently primitive organisms, much finer differentiations between individuals than those which are noted in relatively simple adult metazoan invertebrates, such as Hydra and Planaria, or in the embryos of vertebrates. Although protozoa and the gametes of algae belong to classes of organisms which are considered primitive, it seems that within these classes there have developed, in the course of evolution, very fine differentiations between single cells, which cannot as yet be observed in the organisms from which they are presumably derived. It is therefore possible that in these classes of unicellular organisms mechanisms or substances have evolved, in certain respects analogous to but probably not identical with individuality differentials. The reactions which they manifest are at least partly localized in the ectoplasmic structures of these cells; but inasmuch as the latter may be newly formed by the rest of the protoplasm in a protozoon temporarily deprived of them, we must assume that also other parts of these cells, including perhaps their nuclear substance, have the power to give origin to their own specific individuality differential-like substances.

As we have already mentioned, the observations discussed in this chapter may be of significance also in the analysis of the conditions underlying the formation of colonies which, in some instances, develop from unicellular organisms. Individuality differential-like mechanisms in unicellular organisms tend to keep the individuals separate from other individuals of the same species and thus to insure to those organisms the maintenance of a separate existence. Conversely, it may be concluded that whenever colony formation occurs, reactions characteristic of individuality differential-like substances, such as we have here described, are lacking.

As to the interpretation of the mechanism underlying contact reactions between unicellular organisms, possessing their own individuality or species differential-like mechanisms, Jensen and Verworn started with the assumption that the protoplasm of these cells is liquid throughout. However, from what has been learned since about the constitution of amoeboid cells in protozoa, in amoebocytes of Limulus, and even in cells of higher organisms, it appears that the consistency of the ectoplasmic layer of isolated cells is generally more or less solid, although readily undergoing changes, and that under different conditions its consistency may vary between the extremes of a completely solid and a liquid state. In the case of the organisms under discussion, the contact between the surface layers of two unicellular animals or plants, which latter differ in what in higher organisms would correspond to organismal differentials, may, under certain conditions, act as an abnormal stimulus initiating a softening of the surface layer; this change may be followed by

agglutination and coalescence of the cells, while under other conditions a more complete liquefaction and a subsequent shattering reaction may occur, resulting in the disintegration of the protoplasm into separate droplets in accordance with the alterations in surface tension of the liquids concerned in these reactions.

In the case of the amoebocytes of Limulus it can be shown that numerous environmental changes may produce variations in the consistency of the outer layer of the protoplasm, which in some instances cause agglutination, and in others amoeboid movement. It is therefore conceivable that in other unicellular organisms stimuli, which sometimes lead to agglutination and coalescence, may under different circumstances initiate amoeboid movements of cells in a direction towards each other, influenced in this process by substances which are hormone-like and which may not, themselves, possess organismal differentials. Also, the movements of embryonal cells in the direction towards other tissues may be explained as due to surface reactions similar to those which lead to agglutination, coalescence, or migration in unicellular organisms.

Chapter 2

Tissue Formation and Organismal Differentials

WE HAVE SEEN that incompatibilities between the organismal differentials or their precursors, or between substances analogous to these differentials, but not identical with them, which are present in adjoining cells may prevent the union of the latter and lead to the separation of cells or parts of cells at or near the point where the bearers of the incompatible differentials come in contact. This applies to the union of ova and of embryos, or parts of embryos, in very early stages of development, as well as to the union of free-living, unicellular organisms or parts of them. In other cases it may merely modify the nature of their union. There is a related phenomenon of great biological interest, namely, the formation of tissues through the union of single cells. Here apparently similar factors to those which we have discussed in the preceding chapters are active and it may therefore perhaps be possible to analyze the conditions on which the union of various cells into tissues depends, and to determine whether there is any indication that in this process, also, organismal differentials or related substances play a part.

1. A very simple and primitive type of tissue results from the agglutination of amoebocytes of Limulus, which takes place spontaneously whenever the blood of this animal leaves the body under natural conditions. Because of the primitive nature of this process, it exemplifies, perhaps, some of the principles underlying tissue formation in general, and moreover, it is more readily accessible to experimental analysis than the more complex processes leading to the formation of the fixed tissues in organisms. In contrast to the latter, the amoebocyte tissue is merely an experimental tissue, but the analysis of the factors underlying its formation has served as the starting point for similar studies in the case of the more complex natural tissues.

The essential factor underlying the formation of this amoebocyte tissue is an agglutination process, and the agglutination is due to a change in the environment of these cells, which acts as a stimulus. The stronger the stimulus within a certain range, the greater are the changes in the amoebocytes and the more intense is the agglutination which takes place. Thus, if we make an incision into a Limulus and allow the blood to flow out through such a narrow opening, it will come in contact with the rough surface of the wound and subsequently with the chitinous body covering; under these conditions the amoebocytes send out pseudopods and some of the cells may even change into a diffuse gelatinous material. If the altered cells and the material flowing out from the injured amoebocytes come in contact with one another they stick together, so that they form one jelly-like mass, which gradually retracts into a small *firm* clot, in this respect behaving therefore not unlike a blood coagulum. But if, instead of using this simple process, we collect the blood by means

of a smooth, oiled cannula, in glass dishes kept at a temperature near the freezing point of water, the changes which the cells undergo are much less pronounced, and although under these conditions the cells still agglutinate with one another, the agglutination is less firm, the cells remain preserved much better and gradually sink down to the bottom of the dish, where they form a connected, relatively thin layer of tissue.

However, whether we use the first or second method, in principle we have to deal with the same change in the constitution of the cells. Within the blood-channels of the animal the amoebocyte represents a flat elliptic transparent disc, which is carried along by the blood-lymph current and is not sticky; but under the influence of mechanical and various kinds of chemical stimuli the amoebocyte seems to take up some fluid from the surrounding medium and becomes a round or oval cell with larger granules which are separated by a considerable amount of intergranular substance. As a result of this change in consistency, especially of the outer ectoplasmic layer of the protoplasm, the cells become sticky and adhere to one another as well as to the more or less solid surface of the dish with which they come in contact, or they sink down; furthermore, associated with this change there is a tendency of the amoebocytes to send out pseudopods and to manifest amoeboid movement. These observations suggest that agglutination and amoeboid movement may be related processes. Conditions which tend to increase the consistency of the protoplasm within a certain range, also tend to decrease the stickiness and agglutinability of the cells and to diminish their amoeboid movement. Under the action of these factors the pseudopods become fine, more or less shred-like, and the amoeboid movement is slowed down. Such effects are produced, for instance, by the use of hypertonic salt solutions, by addition of a slight amount of acid to a sodium chloride solution isotonic with sea-water, by an increase in certain ions, as for instance, Na and SO_4, in the surrounding medium, and by exposing the cells to cold. In a limited way, a temporary result of this kind is also brought about by a relatively strongly alkaline NaCl solution. On the other hand, a softening of the cells increases agglutination and, to a certain extent, amoeboid movement; a moderate amount of alkali in an isotonic NaCl solution, hypotonic solutions, an increase in certain ions (K, NH_4, NO_3), and a slight rise in temperature, exert the latter effects. The blood serum of Limulus and extracts of Limulus tissue act in a similar way, and they likewise have a tendency to cause an extension and spreading-out of the amoebocytes on the surface of a glass on which these cells rest. This spreading-out is due to a softening of the cells; it represents a modified type of amoeboid movement, and furthermore, together with the processes which take place during amoeboid movement, it explains the tissue-stereotropism which is common to amoebocyte tissue and to mammalian epidermal and other tissues. In general, all these different modes of reaction of the amoebocytes correspond to variations in the consistency of the protoplasm, and such variations explain the diverse structural types which the cells, singly or combined into tissues, may assume; in addition they explain the modifications in the character of amoeboid movement which may be observed. Moreover,

certain agencies, as for instance, acid dissolved in isotonic NaCl solution within a certain range of concentration, not only diminish agglutination, but may even cause a separation of agglutinated amoebocytes from one another and thus change a tissue-like formation back into a suspension of isolated cells. The agglutination and resulting tissue formation represent, therefore, to a certain extent, reversible processes. A similar reversibility we find also in some of the tissues of the most differentiated vertebrates.

2. With amoebocyte tissue we can imitate and analyze certain phenomena of wound healing which takes place in the normal epidermis of higher organisms. Embedded in this tissue the amoebocytes are at rest, but as soon as an incision is made and a piece cut out, the cells adjoining the wound become active and migrate into the wound, thus tending to cover it. There is a difference in the environment of different parts of the cells adjoining the wound, these cells being in contact with other amoebocytes, on the side away from the wound and with a fluid medium and a glass surface on the side of the defect; and this condition acts as a stimulus, causing amoeboid movement in the direction towards the wound and away from contact with the cells. Similarly, we can excise small pieces of such a tissue, place them on a cover glass, and treat them as we do pieces of higher tissues according to the tissue culture method. Through secondary processes which, under certain conditions, may become degenerative, the character of various higher tissues, especially those of a mesenchymatous nature, may be imitated, and also pictures corresponding to outgrowing fibroblastic tissue may be readily obtained with this experimental amoebocyte tissue. The same factors which are responsible for the movement into the wound of cells and groups of cells adjoining a defect, cause also the active movement of cells in tissue culture. During this process of migration the moving cells meet fresh amoebocytes which are likewise migrating through the culture medium; if they come in contact with one another, they stick together and form small clumps of cells, from which the individual amoebocytes tend to detach themselves again; in this way cell movement takes place, both in tissue culture and in wound healing, in a centrifugal direction, similar to the cell behavior of higher vertebrate tissues under analogous conditions. There is no indication that the movement is otherwise an oriented one; on the contrary, we may consider it as more or less a chance phenomenon.

As stated, it is the physical and chemical changes in the environment which bring about the agglutination of cells and, therefore, those reactions which transform the cells from free-living, isolated cellular organisms into components of tissues. If a corresponding condition existed within the blood channels, as a result for instance of the introduction of a foreign body into the blood, an agglutination would take place here also, which would lead to the formation of an agglutination thrombus consisting of amoebocytes and comparable to thrombus formation in higher organisms, where analogous cells or blood platelets, representing parts of cells, furnish the substratum of the thrombus. Tissue formation and thrombus formation are thus essentially related processes.

While in certain respects amoebocytes and free-living protozoa differ from each other in their behavior as far as amoeboid movement is concerned, there are also some important similarities in these cell types; to mention only one feature common to both: the primary and principal change in the consistency of the protoplasm occurs especially at the point where the pseudopod formation takes place, which is the leading and most active and sensitive part of the cell. Connected presumably in some way with the characteristics of the pseudopods are their fine reactions to individual and species differences, which have been observed in certain protozoa and which we have already discussed. These reactions also depend on changes in the consistency of the protoplasm, especially of the surface of the cells, which take place in accordance with the degree of compatibility or lack of compatibility between the cells which meet; and as we have seen, similar changes are also the principal factors leading to pseudopod formation.

There are, however, also some important differences between amoebocytes and protozoa. In the case of amoebocytes, their behavior, and in particular the degenerative processes they undergo, vary greatly in different media and under different physical conditions. Characteristic of these cells also is their need of a protein medium. The free-living protozoa, on the other hand, are adapted to a medium free of protein. Associated with this difference in the protein requirement of these organisms there is a further difference in their reaction towards certain ions.

As to the possible role substances corresponding to organismal differentials play in the behavior of amoebocytes, there are individual variations observed in the reactions of the cells and consequently also of the amoebocyte tissue derived from different Limuli. Such variations are as a rule, however, manifestations of the quantitatively different tendency on the part of amoebocytes to contract and of associated differences in the consistency of these cells; these result mainly from environmental conditions to which the Limuli have been previously subjected. There seems to be no difference in the behavior of amoebocytes to one another, homoiogenous and autogenous amoebocytes behaving in the same way. Therefore there is no manifestation of an individuality differential or a similar substance noticeable in these cells, as far as their mutual reactions are concerned. They differ in this respect from the protozoa, which we have discussed in the preceding chapter.

The behavior of amoebocytes and the agglutination process leading to a joining together of cells have been considered somewhat more in detail because, as stated, the analysis of experimental amoebocyte tissue shows clearly the principles underlying tissue formation in general, and the union of cells in tissues is the basis of the formation of multicellular organisms. But our conclusions apply only to the granular amoebocytes, such as those of Limulus. The so-called hyaline amoebocytes which have been studied in recent years, especially by Fauré-Fremiet, behave somewhat differently and do not lend themselves to experiments with tissue formation in the same way as the amoebocytes of Limulus.

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observed in free-living protozoa than to those observed in the amoebocytes, which latter are adapted to a protein-containing environment, while the protozoa and sponges are adapted essentially to a medium which consists of a mixture of salts. We may therefore regard the experimental amoebocyte tissue as representing the most primitive and rudimentary type of tissue, and the sponges as the next higher type, in which a further differentiation of the component cells and their power to proliferate are added to the primitive mode of agglutination and tissue formation.

More recently the agglutinated sponge cells have been studied by Fauré-Fremiet in tissue culture in a similar manner to the amoebocyte tissue, and he has shown that the archaeocytes behave, here, in about the same way as the amoebocytes; they move out of the peripheral piece of tissue in a centrifugal direction and flatten out. It seems, also, according to this investigator, that a further development of such a tissue culture into a typical sponge takes place only if the archaeocyte tissue has become agglutinated to the surface on which it has been placed. In both cases the processes leading to agglutination depend on changes in the ectoplasmic layer, which make it sticky, probably as a result of the taking-up of a certain amount of fluid by the stimulated cell. In addition to the archaeocytes, the collencytes and the choanocytes take part in the formation of the complete sponge, while the other structures are produced through differentiation of these primary cells. It seems that the excretory canals are the central organ around which the other structures are built up.

If the amoeboid cells of two different species of sponges, such as Microciona and Ciona, are mixed, two types of reaction may be noted: (a) When separate archaeocytes of Microciona and Ciona come into contact the outer hyaline layers of the protoplasm of the cells belonging to the different species fail to coalesce, the cells of each species remaining separate and forming aggregates of their own kind. Such a segregation is evidently caused by differences in the physical properties of the outer protoplasmic layers of the cells of these two species, and possibly, as in the case of pseudopods of certain protozoa, by specific changes which take place in the consistency of the protoplasm when cells possessing different species characteristics meet. There may also be involved in this effect of foreign cells, either sessile contact substances or substances secreted by these cells, or substances liberated from the cells when they are injured in the preparation of the suspension. We may have, in this case, to deal chiefly with the action of contact substances, which lead to separation of cells if they are heterogenous; in addition there may be, as stated, direct physical differences in the cell membranes, which prevent agglutination and normal tissue formation. In this connection it is of interest to note that, according to Galtsoff and Pertzoff, the cells of Ciona and Microciona differ also in the pH of their cell content. (b) But there may take place a second type of interaction between cells of different species. As a result of the unfavorable effect of substances extracted from a suspension of heterogenous sponge cells, the archaeocytes are injured; rapid cytolysis take place and the outflowing cytoplasm of the degenerating cells agglutinates to form

to the development of colonies out of isolated cells. Such colonies are observed, for instance, in the ciliate *Zoothamnium alternans*; but here, in contrast to the most primitive tissues, a differentiation in function has taken place between different members of the colonies, as Summers has shown. The apical cell exerts an inhibiting effect on neighboring cells. When the apical cell is cut away, a formerly subordinate cell becomes dominant and assumes the generative function of the apical cell. But the latter may also exert a stimulating effect on the other members of the colony. If it becomes an exconjugant, it induces the first three or four branches below its own level to divide precociously and so actively that each branch develops almost as an individual colony. In such a colony evidently a complex tissue equilibrium exists, but whether this equilibrium requires a strictly autogenous relationship between the various members of the colony is not certain.

3. The next higher type of tissue formation is found in sponges. Some complications are added here to the primary factors observed in amoebocytes. H. V. Wilson, who first separated sponge cells experimentally, was able to observe that these cells later united again with one another, forming aggregates from which, under favorable conditions, complete sponge organisms developed. More recently Galtsoff noted that it is the archaeocytes which play the principal role in the agglutination of sponge cells, and that they resemble very much in their behavior the amoebocytes of Limulus. As in amoebocytes, so also in sponges the migrating cells happen to meet other cells of the same kind in the course of their movements, and whenever such a chance meeting takes place the cells stick together. In both cases there is the same lack of an orienting force which leads to the tissue-like agglutination of cells. However, subsequently some differences develop between the behavior of amoebocyte tissue and sponge-cell aggregations. In the latter, a secondary detachment and migration of cells in a centrifugal direction does not occur as it does so often in the former; instead, they now spread out on the surface on which they are resting, in a way comparable to the extension which is such a common occurrence and which we have analyzed in amoebocyte tissue. In both these types of cells the process of extension can be considered as a pathological modification of amoeboid movement. However, subsequently, the aggregates of sponge cells, provided they are sufficiently large and contain cells of a certain type, may change into normal proliferating sponges, while from amoebocyte tissue more complex formations may develop merely as a result of secondary, often degenerative changes which lead to the production of paraplastic structures. The mechanisms, in both instances, underlying the primary agglutination and the development of stickiness in the hyaline ectoplasm, which latter precedes the agglutination process, are related to the factors concerned in the production of pseudopods and in the extension of the cells. Temperature, osmotic pressure and hydrogen ion concentration seem to affect amoebocytes and archaeocytes in a similar manner; for instance, alkali increases the tendency of both kinds of cells to agglutinate. But as far as the effect of salts, and of ions composing them, on tissue formation is concerned, the reactions of archaeocytes apparently correspond more closely to those

is balanced, as far as these cells are concerned, cannot be produced, since they need a protective colloid in the form of protein, as has already been stated in the case of Limulus. However, subsequent investigators (Brian, Ries) attribute the tissue formation during the process of budding, not to these amoeboid cells, but to special cells which resemble more closely lymphocytes and which have a tendency to divide mitotically. Ries assumes that the packages of amoeboid cells which are seen, serve merely as foodstuffs during the process of tissue formation; but even if this view should be correct, still, the amoeboid cells of Clavelina do produce tissue-like formations during or preceding the process of budding and migrate towards the regions where active tissue formation occurs, and in this respect they resemble in their mode of reaction the amoebocytes of Limulus and the archaeocytes of sponges under injurious conditions.

There is some indication that also in other instances the blastema from which regenerative processes proceed, takes its origin from cells migrating to a wound from distant parts of the organism. Observations of this kind have been made by Balinsky and Hellmich, and we have referred to them in a previous chapter. There is, however, some doubt at present as to whether we have to deal in these processes with the migration of more or less undifferentiated mesenchymatous cells possessing great developmental potentialities, or with the migration of already more or less differentiated cells giving rise to the new tissue. If the migration of undifferentiated wandering cells should actually play so great a part in regenerative processes as is assumed by Hellmich, it is quite probable that here, also, agglutination and possibly coalescence of these cells precede tissue formation.

5. Tissue formation which takes place during embryonal life begins with the segmentation of fertilized or parthenogenetically developing ova; but in this case, underlying the union of the cells is a more complicated mechanism. This depends, above all, on the presence of membranes surrounding the ovum and the early embryo, and furthermore, on certain special structures which connect the individual segments. However, the methods which are successful in accomplishing the union of different ova or blastomeres, or in separating normally united blastomeres from each other—both processes being influenced by changes in alkalinity, in Ca content, and in the temperature of the surrounding medium—indicate that also in these cases we may primarily have to deal with agglutination processes due to changes in the consistency of the ectoplasm of ova or blastomeres. These primary changes may then be secondarily followed either by coalescence or by fargoing cell and tissue differentiations. It may be assumed, therefore, that also in the first stages of the formation of multicellular embryos, agglutination processes, not unlike those which occur between amoebocytes of Limulus, may play a significant role.

With this conclusion harmonize also the experiments relating to the agglutination and coalescence of ova and blastomeres in various classes of animals, to which we have referred in a preceding chapter. The organismal differentials or their precursors were found to be an essential factor in de-

a floccular material which likewise gradually becomes dissolved. There is, however, no perfect correspondence between the phylogenetic relationship of the two species and the way in which these heterogenous cells act on each other; furthermore, the results obtained with reciprocal combinations may vary. Whether the cytolytic substances involved in this process differ only in quantity, or also in kind, from the contact substances mentioned above is uncertain. It may be added here that in arthropods we have observed that a precipitation takes place if the sera belonging to different species are mixed with each other; this may be a related phenomenon to the cytolytic effect of heterogenous substances seen in sponges.

4. A still higher type of tissue formation has been described by Spek in the tunicate Clavelina. Within this organism amoebocytes are found carrying special cell inclusions and wandering to places where, owing to the presence of a wound, regenerative or reduction processes occur, such as are associated with bud formation. These amoebocytes migrate in great numbers and either go to the area of new-growth or accumulate in the body cavity. Here they agglutinate to form clumps or masses, arranging themselves in a tissue-like manner, and according to Spek, subsequently giving rise to the formation of the new tissues and organs. Under normal conditions when instead of other amoebocytes they meet cells of a different kind, or if they migrate through other tissue layers, they do not agglutinate with one another nor do they agglutinate with the other kind of cells. But as soon as their environment becomes abnormal, as for instance, near a wound, or when during the reduction processes in the animal they are exposed to conditions under which abnormal products of disintegration act upon them, or when the preformed tissues in these tunicates are unable to undertake the necessary regenerative functions, then these cells become sticky and agglutination occurs. Preceding the formation of clumps under such abnormal conditions, the amoebocytes migrate in masses to areas which presumably have undergone pathological changes, either to the aboral pole in dying animals or into the body cavity prior to the formation of winter buds. In case of regeneration of special organs they may first form epithelium-like surfaces, a process which likewise presupposes agglutination. Smaller groups may then agglutinate with one another, so that larger or sausage-like masses result, but the agglutination processes are always preceded by active amoeboid movement, and this is an oriented one, directed apparently by substances produced in regions where pathological processes take place. These movements and agglutination processes are followed by organ formation.

Thus we note here a close parallelism to the reaction of amoebocytes of Limulus, where likewise abnormal environmental factors cause changes in the surface layer of the cells leading to agglutination and formation of tissue-like layers, but in Clavelina, as well as in sponges, these primary processes are followed by the development of differentiated tissues and organs. There is an additional point of similarity between amoebocytes of Limulus and the amoebocytes of Clavelina; for both of these types of cells sea-water or solutions of inorganic constituents, as such, are toxic, and a salt solution which

is balanced, as far as these cells are concerned, cannot be produced, since they need a protective colloid in the form of protein, as has already been stated in the case of Limulus. However, subsequent investigators (Brian, Ries) attribute the tissue formation during the process of budding, not to these amoeboid cells, but to special cells which resemble more closely lymphocytes and which have a tendency to divide mitotically. Ries assumes that the packages of amoeboid cells which are seen, serve merely as foodstuffs during the process of tissue formation; but even if this view should be correct, still, the amoeboid cells of Clavelina do produce tissue-like formations during or preceding the process of budding and migrate towards the regions where active tissue formation occurs, and in this respect they resemble in their mode of reaction the amoebocytes of Limulus and the archaeocytes of sponges under injurious conditions.

There is some indication that also in other instances the blastema from which regenerative processes proceed, takes its origin from cells migrating to a wound from distant parts of the organism. Observations of this kind have been made by Balinsky and Hellmich, and we have referred to them in a previous chapter. There is, however, some doubt at present as to whether we have to deal in these processes with the migration of more or less undifferentiated mesenchymatous cells possessing great developmental potentialities, or with the migration of already more or less differentiated cells giving rise to the new tissue. If the migration of undifferentiated wandering cells should actually play so great a part in regenerative processes as is assumed by Hellmich, it is quite probable that here, also, agglutination and possibly coalescence of these cells precede tissue formation.

5. Tissue formation which takes place during embryonal life begins with the segmentation of fertilized or parthenogenetically developing ova; but in this case, underlying the union of the cells is a more complicated mechanism. This depends, above all, on the presence of membranes surrounding the ovum and the early embryo, and furthermore, on certain special structures which connect the individual segments. However, the methods which are successful in accomplishing the union of different ova or blastomeres, or in separating normally united blastomeres from each other—both processes being influenced by changes in alkalinity, in Ca content, and in the temperature of the surrounding medium—indicate that also in these cases we may primarily have to deal with agglutination processes due to changes in the consistency of the ectoplasm of ova or blastomeres. These primary changes may then be secondarily followed either by coalescence or by fargoing cell and tissue differentiations. It may be assumed, therefore, that also in the first stages of the formation of multicellular embryos, agglutination processes, not unlike those which occur between amoebocytes of Limulus, may play a significant role.

With this conclusion harmonize also the experiments relating to the agglutination and coalescence of ova and blastomeres in various classes of animals, to which we have referred in a preceding chapter. The organismal differentials or their precursors were found to be an essential factor in de-

termining whether such an agglutination will or will not take place and whether the union will be temporary or permanent. Similar factors, and in particular agglutination processes, may perhaps be concerned also in the joining together of parts of more primitive adult organisms, such as Hyla, Planaria, Lumbricus, and even in the transplantation of extremity buds in Triton, or of extremities in the larvae of Salamander, although the processes underlying these latter phenomena have not yet been analyzed from this point of view.

6. Even in still higher organisms, as, for instance, in mammals, when a wound is made in the epidermis, reactions follow, not unlike those observed in experimental amoebocyle tissue, and in all probability the factors underlying both these phenomena are likewise similar. However in the more differentiated tissues more complex structures, which connect neighboring cells or tissues to one another, have developed, and these may vary in the different tissues; but even in the mammalian skin these complex structures disappear during wound healing, at least temporarily, and then the primitive reactions, which are common to so many organisms and which we have analyzed in this chapter, have a chance to set in. In addition, we have reason for assuming that in the tissues of higher animals there are at work finely graded substances *carrying individuality differentials* and *regulating the* interaction of tissues of the same type, as well as of different types adjoining each other within the same organism. These autogenous morphogenic regulators have already been discussed.

It may therefore be assumed that also tissue cells of higher organisms still possess the fundamental properties of amoebocytes, at least potentially, and that only secondarily other, more complicated structures and functions are superimposed upon these primary characteristics and that especially in certain artificial or pathological conditions, such as those leading to wound healing, these primary modes of reaction come again into play. But there are found even in higher organisms certain types of cells which, within the normal organisms, remain isolated; among them are the erythrocytes, especially the nucleated ones, the various types of leucocytes and the spindle cells, which as far as their function is concerned, take the place of mammalian blood platelets in other vertebrates. These, as well as the blood platelets, possess in various degrees the characteristics of the amoebocytes. The tendency to agglutinate is most markedly developed in the avian spindle cells of the blood and in the mammalian blood platelets, but it is to a lesser degree also found in the other types of cells. In all these elements, and particularly in the spindles and blood platelets, the stickiness of the outer cell layer is lacking under normal conditions within the blood channels. It is only under the influence of abnormal stimulation that their protoplasm undergoes changes which, in principle, are presumably similar to those we have analyzed in the case of amoebocytes, and which lead to agglutination and thrombus formation, or to tissue or cell reactions of a so-called inflammatory kind. As to the rôle which organismal differentials play in these latter processes, no definite knowledge exists.

Chapter 3

The Role of Organismal Differentials in Fertilization

IN PRECEDING chapters we have considered the joining together of protozoa, ova, blastomeres, amoebocytes, and more differentiated tissue cells, in their relation to organismal differentials, and we found that in general agglutination processes play a significant part under these various conditions. A somewhat similar process takes place when two cells unite in the fertilization of an ovum by a spermatozoon, although the interaction between these two cells is of a more complicated nature.

It was O. Hertwig who first drew attention to the similarity which exists between the process of fertilization and transplantation, fertilization being considered as the transplantation of a spermatozoon into an ovum. He designated the relationship between these cells, upon which their mutual compatibility depends and which may vary in different combinations, as sexual affinity, and distinguished it from the vegetative affinity which determines the relationship between somatic transplants and hosts. Later, W. Schultz, who, within a limited range, carried out investigations concerning the relationship between transplantability and genetic conditions, tested experimentally the question as to whether a parallelism exists between transplantability of parts of heterogenous individuals and the feasibility of hybridization between the same host and donor species.

However, although in certain respects transplantation and fertilization are analogous processes, there are also some essential differences which had not yet been clearly recognized by Hertwig. While in both processes the result depends upon the relationship between two organisms or parts of organisms, the kind of genetic relationship which is normal or optimal is not the same in both cases. Whereas the relationship between spermatozoon and egg is usually homoiogenous, and this is the one best suited for a successful fertilization, such is not the case in transplantation of adult tissues in higher organisms. Here a homoiogenous relationship between the organismal differentials of host and graft leads, as a rule, to severe reactions and to injury of the transplant. Furthermore, in transplantation of differentiated tissues in higher adult hosts we have to deal with the interaction of two fully developed organismal differentials, whereas in fertilization we have to deal, in the main, with the transplantation of nuclear material, and especially of the chromosomes of the spermatozoon, into a cell which likewise does not yet possess a fully developed organismal differential, nor the mechanism by means of which differentiated organisms react against strange organismal differentials. Ovum and spermatozoon each carry a substance or substances which later in the course of embryonal development will give origin to a fully formed

organismal differential, identical in various organs and tissues of the same individual and species.

In many instances where, in plants and animals, both male and female germ cells are produced in the same organism, mechanisms of a special kind have developed, tending to prevent autofertilization, which otherwise would have been the simplest mode of fertilization but which might have injurious consequences. Even syngenesio-fertilization occurring in succession through *many generations leads* in many cases to a gradual deterioration of the organism.

On the other hand, if *heterofertilization* takes place, incompatibilities also develop, as a rule, sooner or later, even if spermatozoon and egg belong to relatively nearly related species; but *these incompatibilities may in certain* respects be less marked than those in heterotransplantation and the spermatozoon may even, under such conditions, remain alive and apparently unharmed in the strange ovum, whereas, after transplantation of differentiated tissues between the corresponding two species in mammals the host reacts very strongly against the transplant, which is severely injured and, as a rule, destroyed within a relatively short time. Thus in Echinoderms, by means of fertilization between different orders it is possible to produce hybrid plutei, which in certain of their characteristics are intermediate between the two parent orders. In some instances, a slight increase in the constitutional differences between spermatozoon and egg above those characteristic of the average homoiogenous relationship between the organisms which carry the sex cells, may even have a stimulating effect on the developmental processes resulting from fertilization and may thus prove favorable at least in the first generation. But in the case of transplantation the incompatibilities between transplant and host, and the resulting injury of the transplant, increase rapidly with increasing strangeness of the organismal differentials.

However, notwithstanding these differences between fertilization and transplantation, there is one very essential similarity; after heterogenous fertilization as well as after heterogenous transplantation incompatibilities do, as a rule, develop, which to a certain extent are the greater, the greater the differences in the constitution of the organismal differentials or of their precursors in the cells or tissues which are joined together. A markedly heterogenous character of the precursor substances of the organismal differentials in egg and spermatozoon is associated with an abnormal interaction, causing an interference with the development of the resulting hybrid; but with less incompatible precursors of organismal differentials the development may continue long enough for a specific organismal differential to form in the hybrid, which thus acquires its own mechanism of reaction against strange organismal differentials.

As to incompatibilities developing between spermatozoa and ova, which are sufficiently distant genetically from each other, these, in general, may be caused by two factors: (a) an incompatibility between the spermatozoon and the surface layer of the strange ovum; (b) incompatibilities between the nuclei of these two cells, and in particular between their chromosomes, or

between the sperm nucleus and the cytoplasm of the ovum. The second type of abnormal interaction represents, on the whole, much the finer test for the mutual fitness of the interacting cells. Thus the spermatozoon may readily enter the ovum when the distance of the two partners in the spectrum of relationship is not too great, but subsequently, incompatibilities between the cells, and especially between their nuclear constituents, may manifest themselves, or the spermatozoon may be entirely inactivated or eliminated from the ovum, so that a parthenogenetic development of the stimulated egg takes place.

But there exists within certain limits, in addition, a proportionality between the difficulty which the spermatozoon experiences in entering the egg and the distance in relationship between these two cells. If the distance is very great, for example, when ovum and spermatozoon belong to different classes, it is necessary to make the surface of the egg more sticky by treating it with alkali, according to the method of Jacques Loeb, or by allowing the egg to become stale in order to effect the entrance of the spermatozoon into the ovum. In certain echinoderms, making a dense suspension of eggs, without first washing them in sea water, seems to improve the results in heterofertilization (E. Browne Harvey). It is, then, only after this difficulty has been successfully overcome that the more serious antagonism between the constituents of the male and female germ cells becomes manifest. If the distance in relationship between egg and spermatozoon is very great, the paternal chromatin is prevented from orderly interaction with the egg chromatin; instead, it is pushed aside into the cytoplasm of the ovum. This reaction may take place almost at once, or it may occur later, during the process of segmentation. The subsequent development is, under these conditions, parthenogenetic. However, even such a development is not normal; it appears as though the mere presence of the strange chromatin in the ovum exerts an injurious effect on the latter. Either the development of the embryo may be merely retarded, or certain abnormalities in differentiation may occur and the resulting organism may therefore be less viable than a normal one. Furthermore, such organisms, if they should reach the larval stage, do not usually undergo normal metamorphosis.

From conditions of marked incompatibility between egg and spermatozoon, we find all degrees of transition, to an almost complete harmony between these cells. If in an intermediate stage there is a mild degree of disharmony, only a part of the paternal chromosomes may be eliminated and in the resulting embryo the maternal characteristics may predominate over the paternal. As Baltzer and Tennent have shown, elimination of chromosomes may occur at different stages of development: as early as during the first segmentation or later in the blastula stage. According to Tennent, not only paternal but also maternal chromosomes may be eliminated. On the other hand, if sperm and egg are so far removed from each other as to belong to different classes, the paternal chromatin may in some cases be cast out even before the first segmentation. All kinds of irregularities or monstrosities can be observed under these conditions, and in general they are the more severe,

the less compatible the interacting germ cells. As an example of a slight degree of disharmony, we may cite the observation of Doncaster and Gray in crosses between different species in echinidae, who found, as the only abnormality, a vesicle formation on the part of a few chromosomes.

Disharmonies between the chromatin of egg and spermatozoon can also be produced experimentally. Thus as a result of injury to the chromatin of spermatozoon or ovum, caused by the exposure of these cells to radiations or to certain dyes preceding fertilization, the result of homoiofertilization can be made to resemble that of heterofertilization. Incompatibilities develop here between the precursors of the organismal differentials of the two germ cells, or between the carriers of these precursors. These incompatibilities may be so severe that the injured nucleus no longer participates in the development and parthenogenesis results, similar to that found in hybridization between different classes. However, in case the injury of the chromatin has been less pronounced, the radiated nuclear substance may cause merely abnormalities in embryonal development.

There exist certain critical periods in embryonal life when difficulties in the formation of some tissues and organs are especially prone to arise, as for instance, during the process of gastrulation; in addition, it is conceivable that a summation of injurious effects takes place gradually as development proceeds. Furthermore, the incompatibility may affect either growth and differentiation or viability, or both jointly, and there may be a parallelism between the retardation in growth and in the abnormalities in differentiation of the embryo as a whole, or of its individual organs, resulting from heterogenous fertilization.

The following list of the results of heterofertilization in echinoids, which Tennent gives, may show the gradation of injurious effects in a certain order of animals.

1. Elimination of no chromosomes and dominance of one species with inactivation of incompatible chromosomes:
   Toxopneustes ♀ X Hipponoë ♂ (different genera)
   Echinus ♀ X Antedon ♂ (different families?)
   Strongylocentrotus ♀ X Antedon ♂ (different families?)
2. Elimination of part of chromosomes and dominance of one species over the other:
   Hipponoë ♀ X *Toxopneustes* ♂ *medium incompatibility* (different genera)
   Echinus ♀ X Sphaerechinus ♂ (different families)
   Strongylocentrotus ♀ X Sphaerechinus ♂ (different genera)
3. Elimination of no chromosomes and intermediate plutei:
   Sphaerechinus ♀ X Strongylocentrotus ♂ (different genera)
   Sphaerechinus ♀ X Arbacia ♂ : most compatible (different suborders)
4. Elimination of part of chromosomes and intermediate plutei:
   Toxopneustes ♀ X Hipponoë ♂ (different genera)
   Arbacia ♀ X Echinus ♂ (different suborders)

5. There may be elimination of part of both maternal and paternal chromosomes and inhibition of development. Unfavorable.
   Arbacia ♀ X Toxopneustes ♂ (different suborders)
   Toxopneustes ♀ X Arbacia ♂ : fairly compatible (different suborders)

There is, here, at least an indication of a parallelism, although not a complete one, between relationship and the results of heterofertilization. The best results were obtained in the case of hybridization of suborders. In these experiments we notice differences between the reactions in reciprocal heterofertilizations. Differences in reciprocal hybridizations were noted also in fertilization between different orders of echinoderms (Echinocyanus and Parechinus), when plutei, in some way intermediate between both parents, could be obtained if certain combinations were used. Similar differences we have observed also in the case of transplantation; even in this respect there is thus a correspondence between transplantation and hybridization.

Of special interest are the hybridizations between Drosophila melanogaster and Drosophila simulans, because in these insects the genetic constitution of the two parent species has been analyzed very carefully, primarily by genetic methods, and more recently by a cytological study of the chromosomes. Sturtevant found that in both these species, the second and X chromosomes contain the same genes and that the latter are arranged in the same order, but D. simulans has a long inversion in the right limb of chromosome 3, as compared to melanogaster. Certain variations between these two species pertain to the different distances between the genes in the corresponding chromosome, also to the different lengths of the Y chromosomes and to the relative amounts of heterochromatin in the X chromosomes.

Examination of somatic cells in the hybrids between Drosophila melanogaster and Drosophila simulans suggests the possibility that differences in certain genes prevent, here, the normal union of homoiogenous chromosomes. Even such slight differences in gene composition as exist between these two species and the resulting incomplete union of chromosomes, lead to sterility in the hybrid. Other species of Drosophila cannot be hybridized, presumably because of the greater differences in gene constitution. If, however, crosses are made between still more nearly related organisms, as for instance in the experiments of Lancefield, who hybridized two races of Drosophila pseudoobscura, abnormalities of a lesser degree may arise, especially during the process of crossing over, but sterility results if in certain chromosomal loci the alleles are derived from the two races.

In the hybridization between species as nearly related as horse and donkey, incompatibilities occur during meiosis in the male sex cells of the hybrid, and in the primary spermatocytes of the F_1 generation abnormal mitoses appear. A lack of coordination in the action of chromosomes derived from these different species leads to disturbances. Under certain conditions even lymphocytes may be attracted by the abnormal substances which are presumably present at later stages of the development of tissues in such hybrids.

Differences in the results of reciprocal fertilization are very evident in the

experiments of Montalenti, who crossed Bufo viridis and Bufo vulgaris. In this case the combination Bufo viridis ♀ X Bufo vulgaris ♂ was much more unfavorable than the reciprocal combination, Bufo vulgaris ♀ X Bufo viridis ♂. In the former type of hybrids retardation in development and abnormal morphogenesis may affect early cleavages and gastrulations; subsequently, malformations appear, especially in the development of the nervous system and of the heart. In the reciprocal crosses, alterations in early stages of development, resulting in the death of the embryos, are very rare. The large majority of these embryos develop like the controls, although at first there may be some delay in development. The tadpoles seem to be about normal, but later on, during metamorphosis, there is a considerable mortality.

This difference in the results of reciprocal hybridization, as in those of transplantation, may be attributed to the dissimilar role which host and donor play in these processes; in hybridization it is the ovum which acts as host to the spermatozoon which it receives into its body. The dissimilarities in the significance of egg and spermatozoon may be taken to indicate that it is not only the chromosomes of these two cells which interact with each other, but that the chromosomes of the male germ cells interact also with the cytoplasm of the egg.

In accordance with the more complex and delicate chemical differentiation of cells and tissues, which progressively takes place during the development of the embryo, the interaction of the chromosomes derived from the two parents evidently becomes, correspondingly, a process of increasing delicacy, and finer differences in the relationship between sperm and ovum may therefore, as a general rule, manifest themselves only during the later developmental periods, while coarser differences may result in abnormalities at much earlier embryonal stages.

It appears that in some of these incompatibilities processes of a purely mechanical character may be involved, as, for instance, maladjustment in the size and shape of male and female chromosomes, or in the movements of the asters and chromosomes, and, somewhat later, disturbances in the rhythms of mitotic divisions and in the developmental rhythms characteristic of the paternal and maternal species in general may interfere. However, there is reason for believing that in hybridization interactions of a chemical nature between substances derived from the male and female germ cells may also be of importance; and in this respect, again, the mechanisms active in transplantation and in fertilization would then resemble each other. These chemical interactions may be of a toxic nature, if the two individuals from which the germ cells are derived do not belong to the same species; toxic effects of this kind have been suggested by Jacques Loeb and Moenkhaus. But it seems that in some cases, in which the distance in relationship between the male and female cells is very slight, the offspring may not only not be defective, but embryonal development may, on the contrary, be accelerated; observations of this kind we shall discuss in the next chapter. Also in the hybrids, Bufo vulgaris ♀ X Bufo viridis ♂, to which we have referred above, it was found by Montalenti that there were some tadpoles, a few weeks old, which ac-

quired a larger size, underwent metamorphosis earlier and exhibited lower mortality than the controls. In such instances of heterofertilization conditions apparently exist comparable to those characteristic of homoiogenous fertilization; but, in addition, certain differences between the germ cells may exert a stimulating effect which is favorable instead of being injurious.

While, then, within certain limits there is presumably a proportionality between the incompatibilities which develop in hybridization and the distance in relationship between egg and spermatozoon, exceptions to this rule do occur and may be very striking. They are most likely due to the presence of secondary factors superimposed upon the primary ones, which latter would act in accordance with the greater nearness or distance of relationship between spermatozoon and egg. Thus the degree of resistance to injurious conditions on the part of these cells belonging to two different species may vary in different cases, irrespective of phylogenetic factors.

There is another fact indicating the lack of complete correlation which may be present, in certain respects, between readily effected hybridizations in different species and the phylogenetic relationship between egg and spermatozoon. It seems that in certain cases heterogenous fertilization may succeed as well in teleosts as in echinoderms, although the latter stand much lower in the phylogenetic scale than the former. Conditions are different in transplantations; here we find, as a general rule, that transplantability becomes more and more restricted with increasing phylogenetic development. In plants, on the other hand, transplantations seem to succeed over a wider range of phylogenetic relationships than hybridizations.

It may then be concluded that while there exist distinct similarities between transplantation and fertilization, there are also notable differences. To recapitulate some essential facts: In both these processes we have to deal with what may be considered a host-donor relation, the ovum representing the host and the spermatozoon the donor cell in the case of fertilization. In both processes the host has a function which differs from that of the donor and in both the reactions of the host preponderate; however, on the whole the constitution and function of the spermatozoon are of a relatively greater significance for the fate of the host, in the case of fertilization, than is the piece of grafted tissue or organ for the recipient organism in the case of transplantation. While in transplantation in adult mammals it is the character of the organismal differentials in host and donor which is the most important factor determining the fate of the transplant, in the interaction between ovum and spermatozoon we have to deal with the precursors of organismal differentials; while in transplantation it is the autogenous relationship between the organismal differentials which is most favorable for a satisfactory interaction between transplant and host, in fertilization it is the homoiogenous relationship which may be considered normal and, as a rule, conducive to the best results. But, if we compare fertilization with transplantation between embryonal organisms, the difference between these two processes is less pronounced, inasmuch as also in embryonal grafting we have to deal with precursors of organismal differentials and not with fully developed organ-

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It may then be concluded that while there exist distinct similarities between transplantation and fertilization, there are also notable differences. To recapitulate some essential facts: In both these processes we have to deal with what may be considered a host-donor relation, the ovum representing the host and the spermatozoon the donor cell in the case of fertilization. In both processes the host has a function which differs from that of the donor and in both the reactions of the host preponderate; however, on the whole the constitution and function of the spermatozoon are of a relatively greater significance for the fate of the host, in the case of fertilization, than is the piece of grafted tissue or organ for the recipient organism in the case of transplantation. While in transplantation in adult mammals it is the character of the organismal differentials in host and donor which is the most important factor determining the fate of the transplant, in the interaction between ovum and spermatozoon we have to deal with the precursors of organismal differentials; while in transplantation it is the autogenous relationship between the organismal differentials which is most favorable for a satisfactory interaction between transplant and host, in fertilization it is the homoiogenous relationship which may be considered normal and, as a rule, conducive to the best results. But, if we compare fertilization with transplantation between embryonal organisms, the difference between these two processes is less pronounced, inasmuch as also in embryonal grafting we have to deal with precursors of organismal differentials and not with fully developed organ-

experiments of Montalenti, who crossed Bufo viridis and Bufo vulgaris. In this case the combination Bufo viridis ♀ X Bufo vulgaris ♂ was much more unfavorable than the reciprocal combination, Bufo vulgaris ♀ X Bufo viridis ♂. In the former type of hybrids retardation in development and abnormal morphogenesis may affect early cleavages and gastrulations; subsequently, malformations appear, especially in the development of the nervous system and of the heart. In the reciprocal crosses, alterations in early stages of development, resulting in the death of the embryos, are very rare. The large majority of these embryos develop like the controls, although at first there may be some delay in development. The tadpoles seem to be about normal, but later on, during metamorphosis, there is a considerable mortality.

This difference in the results of reciprocal hybridization, as in those of transplantation, may be attributed to the dissimilar role which host and donor play in these processes; in hybridization it is the ovum which acts as host to the spermatozoon which it receives into its body. The dissimilarities in the significance of egg and spermatozoon may be taken to indicate that it is not only the chromosomes of these two cells which interact with each other, but that the chromosomes of the male germ cells interact also with the cytoplasm of the egg.

In accordance with the more complex and delicate chemical differentiation of cells and tissues, which progressively takes place during the development of the embryo, the interaction of the chromosomes derived from the two parents evidently becomes, correspondingly, a process of increasing delicacy, and finer differences in the relationship between sperm and ovum may therefore, as a general rule, manifest themselves only during the later developmental periods, while coarser differences may result in abnormalities at much earlier embryonal stages.

It appears that in some of these incompatibilities processes of a purely mechanical character may be involved, as, for instance, maladjustment in the size and shape of male and female chromosomes, or in the movements of the asters and chromosomes, and, somewhat later, disturbances in the rhythms of mitotic divisions and in the developmental rhythms characteristic of the paternal and maternal species in general may interfere. However, there is reason for believing that in hybridization interactions of a chemical nature between substances derived from the male and female germ cells may also be of importance; and in this respect, again, the mechanisms active in transplantation and in fertilization would then resemble each other. These chemical interactions may be of a toxic nature, if the two individuals from which the germ cells are derived do not belong to the same species; toxic effects of this kind have been suggested by Jacques Loeb and Moenkhaus. But it seems that in some cases, in which the distance in relationship between the male and female cells is very slight, the offspring may not only not be defective, but embryonal development may, on the contrary, be accelerated; observations of this kind we shall discuss in the next chapter. Also in the hybrids, Bufo vulgaris ♀ X Bufo viridis ♂, to which we have referred above, it was found by Montalenti that there were some tadpoles, a few weeks old, which ac-

Chapter 4

Self Fertilization and Autogenous Transplantation

IN THE PRECEDING chapter we have compared the effects of heterogenous and homoiogenous fertilization and transplantation. We shall now consider the significance of self-fertilization, which, if continued through successive generations, may lead in the end to a very great similarity or to identity in the genetic composition of spermatozoa and ova; we shall also consider the influence of close inbreeding, a process which may have a similar effect as far as the genetic composition of egg and spermatozoa are concerned. In order to indicate the analogy between these two processes and the corresponding types of transplantation, self fertilization may be designated as autogenous fertilization, because in this case the two germ cells have developed in the same individual, and fertilization between nearly related individuals may be designated as syngenesiofertilization. Close inbreeding implies serial syngenesious fertilization, and through such brother-and-sister matings a relationship may be attained between members of the inbred group, which, while still in the syngenesious region of the spectrum of relationships, may at last approach an autogenous condition.

As we have seen, genetic factors largely determine the character of the precursors of the organismal differentials in the embryo, as well as of the fully developed organismal differentials, which latter control the interaction between adult tissues; genetic factors play a part also in the interaction between egg and spermatozoon. In addition to these two kinds of interaction there exists a third type, which, while not identical with these, possesses some of the characteristics of both. This third type is represented in the fertilization process in some higher plants. Here we have to deal neither with the direct interaction of spermatozoon and egg, nor with that of two different tissues, but with the interaction of a structure associated with or containing the male germ cell, the pollen, with a specialized tissue which surrounds the egg, and ultimately, with the egg itself. During this process, mechanisms may be active which prevent self-fertilization, when both ovum and spermatozoon originate in the same individual. In plants, incompatibility between pollen-tube and tissues which surround the female sex cell may likewise prevent a successful fertilization if ovum and pollen belong to different species or to different varieties. However, in the case of heterofertilization the pollen-tube may, in some instances actually reach the eggule, but even then a successful fertilization may be prevented, owing to incompatibilities between nuclear or cytoplasmic constituents of the germ cells. We must then consider the spectrum of compatibilities and incompatibilities under the following three conditions, (1) in the interaction of tissues after transplantation,

ismal differentials. However, in these embryonal transplantations it is again the autogenous relationship between host and graft which, on the whole, results most readily in a harmonious combination, although in many cases no distinct difference between an autogenous and a homoiogenous relationship can be noticed. In certain respects the analogy between fertilization and transplantation between single cells, or parts of single cells, such as the grafting of pseudopods in protozoa, would seem to be greater than that between fertilization and transplantation of tissues in higher organisms, but again, comparisons show a very striking difference, insofar as here, also, only an autogenous relationship between cells or parts of cells leads to a satisfactory union, while, as stated, the homoiogenous relationship is, as a rule, the normal one in fertilization.

In addition to other similarities between transplantation and fertilization there is the observation that a reciprocal relationship between the individuals or species serving as host and transplant, and between male and female gametes, may lead to very different results; the latter are due to the differences which exist in these processes between the function of the host and the ovum, on the one hand, and the donor tissue and spermatozoon, on the other hand. In both these processes the phylogenetic relationship between the interacting cells or tissues is a factor which helps to determine the outcome, but the parallelism between such a relationship and the results is, on the whole, much less evident in fertilization than in transplantation.

Moreover, the reactions between the sex cells depend on very special mechanisms which regulate the interaction between spermatozoon and egg, and which are lacking in other primitive cells. Such mechanisms consist in the specific functioning of the chromosomes of male and female sex cells, in the relation of chromosomes to each other and to the cytoplasm of the ovum and in the interaction of certain genes. As a rule, these interactions are optimal in case of homoiofertilization and there may even exist in certain instances means for preventing autofertilization. However, as in transplantation, so also in fertilization, heterogenous relationships between the chromosomes of two parent strains lead to incompatibilities, which are greater if distant species, than if nearly related species, or different races of the same species, are combined.

at the time of the reduction division; but in the male the pollen-tube corresponds not to a diploid somatic tissue, but to a gamete in which there has occurred a reduction division, causing a segregation, and some pollen-tubes possess, therefore, S_1, while others possess S_2. In other plants S_3 and S_4, or S_1 and S_3, may substitute for S_1 and S_2. Now, fertilization is possible if the substance which characterizes the pollen-tube is not present in the female style, otherwise an inhibition in the downgrowth of the pollen-tube takes place and fertilization is prevented; in other words, fertilization cannot follow if the stimulating effect on the pollen-tube, which is due to a difference in the substances characterizing pollen-tube and style, is lacking.

The fertilization experiments which East carried out yielded results which were in agreement with his assumption. There are involved in this case, conditions in which the response of certain tissues to each other depends upon the presence of the same, or of two different substances, in the interacting cells and tissues; when these substances are identical the outcome of the reaction is unfavorable.

If a very large number of various kinds of homoiogenous fertilization experiments are carried out, many different factors can be made to interact with each other and a variety of combinations occurs. It is thus found that if two individuals which differ in a factor are crossed, the F_1 hybrids are fertile with each of the parents. As far as the behavior of the F_1 hybrids towards one another is concerned, they can be placed in four groups, the members of each group being sterile with the other individuals of the same group, and fertile with all members of the other groups.

However, in some species of Nicotiana self-fertilization leads to fertile progeny; this is so in Nicotiana Langsdorfii. Here, possession of the same factors by style and pollen-tube does not interfere with the rapidity of downgrowth, while in Nicotiana alata it leads to sterility.

As to the mechanisms underlying self-sterility, in Medicago sativa, after self-pollination, not only does the pollen-tube grow into the ovary more slowly, but also the number of eggs which are fertilized is smaller; in a number of instances the pollen-tube does not enter the micropyle of the ovulum, and if fertilization should take place, abortion occurs rather frequently. More recent investigations indicate that in certain instances, as presumably also in some other plants, self-sterility is genetically determined and depends upon the presence in pollen and ovule of two recessive genes, which must be present in double dose in order to insure self-sterility. These genetic factors determine the mechanism which causes a very slow rate of downgrowth of the pollen-tube into the style; in the latter, it may also induce the formation of a separating wall, preventing the further movement of the pollen-tube towards the ovule. According to Yasuda, an ovarian secretion diffuses into the style and has this inhibiting effect on the pollen-tube; in addition, this substance may inhibit the germination of the pollen. The ovarian product may also exert an inhibition on otherwise non-self-sterile pollen. According to Eysh, spraying of the flowers of self-sterile plants with alpha naphthalene acetamide directly before or after self-pollination neutralizes this ovarian secretion and

(2) in the interaction of germ cells with each other, and (3) in the interactions of germ cells and of structures associated with the germ cells.

I. In higher plants, Jost (1907) assumed that the retardation in the growth of the pollen-tube into the style, which is observed in certain cases of self-fertilization, was controlled by substances given off by the tissues along which the pollen-tube grew on its way towards the ovary, and which he assumed to be specific for each individual. But, according to Jost, it was not necessarily differences in the chemical constitution of these substances, but differences in the concentration of the latter, which distinguished the different individuals and might suffice to explain their specific effects.

Subsequently, Correns (1912) interpreted the observed facts in accordance with the concepts of Mendelian heredity, and in particular, with the concepts of the pure lines of Johannsen. He investigated self-sterility in Cardamine pratensis and concluded that the inhibiting substances, which in this case do not permit the pollen-tube to penetrate into the style and which thus prevent autofertilization, are not characteristic of the individuals as such. According to this investigator, it would not be correct to assume that a certain substance is unique and occurs only in one particular individual and that it is lost when this individual dies, but he believes that there are substances characteristic of certain pure lines, which are dependent upon the inherited genetic constitution of these lines. However, the individuals in Cardamine do not represent members of pure lines, because each individual is the result of preceding fertilizations in which members of different lines entered. A special combination of substances rather than one particular substance is therefore characteristic of each individual. But according to the law of chance, it is possible even for different nonrelated individuals to possess the same combination of substances. These conclusions of Correns are based on the analysis of the behavior of individuals belonging to the F_1 generation, which develops after fertilization between two homoiogenous individuals of Cardamine pratensis. He carried out back-cross fertilizations between the F_1 hybrids and each of the two parents and thus he could establish the existence of four classes of individuals according to the character of the substances retarding the growth of the pollen-tube, which each one of these individuals possessed. When the father had the factors Bb and the mother the factors Gg, these multiple allelomorphs were transmitted to the offspring according to the rules of Mendelian inheritance. Whenever either B or G, or both together, are present in the pollen and in the female tissues, the pollen-tube is inhibited in its downgrowth. Accordingly, there is only one of the four classes of F_1 hybrids which is fertile with both parents, and it has the factors Bg.

The genetic constitution which causes self-sterility has been subsequently analyzed by East in Nicotiana. East assumes that within the same organism, in the male as well as in the female apparatus, which latter corresponds genetically to the sporophyte and represents therefore diploid somatic tissue, two characteristic substances, S_1 and S_2, exist. In the female the stigma and style retain both these substances, because their segregation is effected only

As to the causes of these differences between transplantation and fertilization, we may consider the following facts: Correns as well as East, in their analysis of self-sterility, compared the results of various combinations, some of which correspond to a syngenesious, others to a homoiogenous relationship. They observed the behavior of sperm and egg, or of pollen-tube and style, toward each other in individuals closely related, as well as in those not closely related though belonging to the same species. In the case of fertilization, the individuals belonging to the same family could be arranged in a few groups in such a way that all the members of the same group behaved in an identical manner, whereas in the case of transplantation various gradations could be found in syngenesious reactions, ranging from those seen in autogenous, to those seen in homoiogenous transplantations.

There remains still to be considered a third difference between transplantation and fertilization. While after autotransplantation antagonistic reactions between host and transplant in higher organisms are lacking, in syngenesiotransplantation they occur sooner or later if sensitive tissues are used. On the other hand, in some experiments in plants fertilization between members of the same group (syngenesious fertilization) and self-fertilization were equally unsuccessful, while fertilization between individuals belonging to different groups did succeed. However, a condition corresponding to what we find in auto- and syngenesiotransplantation in mammalian tissues, has been observed by Correns also in the case of fertilization in a plant, namely, in Tolemiea Menziesii. Here, individuals of the F_1 generation can be readily fertilized by one another, as well as with both parents, whereas self-fertilization is impossible. This may be considered as another type of self-sterility and it corresponds to what we find in transplantation if we choose, for instance, the thyroid gland as a test object, and the presence or absence of a reaction as the standard for measurement. In transplantation as well as in the case of fertilization in Tolemiea, it appears that a greater number of factors is required than in the other instances of pollination, mentioned above, in order to explain the results in accordance with the rules of Mendelian heredity.

In hetero-pollination, including pollination between different varieties as well as between different species, there may develop disharmonies of various kinds, which may be similar to those observed in attempted self-fertilization. Thus, lack of germination of the pollen-grain and inhibition in the downgrowth of the pollen-tube into the style may be noted in both cases; likewise, in hetero-pollination there may be in addition an interference with those mechanisms which direct the movement of the pollen-tube through the micropyle toward the ovulum. It seems, therefore, that the specific chemotropically active substances, which function under these conditions, are not interacting in a normal manner with the pollen-tube. The relationship between substances of this kind and the pollen-tube is evidently a specific one, which is graded in accordance with the genetic relationship between the interacting organisms, and these substances behave, therefore, in this respect, in a manner similar to the organismal differentials of the tissues in higher organisms.

But if hetero-fertilization should actually take place, leading to the produc-

thus makes self-fertilization possible. There are also other methods which have such an effect; the most interesting one of these is, perhaps, the induction of polyploidy by means of colchicine treatment of some branches in Petunia axillaris (Stout and Chandler). Self-compatibility was procured for all seedlings obtained from the self-fertilized flowers of tetraploid branches. Moreover, these seedlings could be cross-fertilized; likewise, backcrosses to parents were fertile, except the combination of a tetraploid female seedling and a diploid male parent. The genetic balance is presumably changed in these tetraploid plants in such a way that the mechanism controlling the movement of the pollen-tube towards the ovule is no longer inhibited. How many genes are involved in this process in polyploid organs is not known.

If we compare conditions in transplantation of mammalian tissues with those in pollination experiments, we find certain analogies. The results in both processes depend upon whether certain substances are the same or are different in the two interacting cells or cell complexes. This determines the relations between host and transplant, as well as those between spermatozoon and female sex apparatus. In both cases the reactions are more intense in a homoiogenous than in an autogenous or syngenesious relationship, but homoiogenous reactions injure a transplant, whereas they are as a rule beneficial in pollination. Furthermore, in transplantation we noticed in certain instances marked differences in the types of reaction resulting from a reversal of the relationship between transplant and host, and in a similar way, differences were observed in certain plants in the case of reciprocal fertilization. There is another observation which is of special interest because it corresponds to certain findings in transplantation. If the same plant is pollinated by two different types of pollen, each one behaves in its own way, uninfluenced by the presence of the other. In a similar way we have found that in case of simultaneous transplantation of pieces of tissues from two different donors into the same host, the specific reactions, as determined by the mutual relationships between the individuality differentials of the various transplants and the host, take place around each transplant in their characteristic manner, without any influence of the other transplant being noticeable.

However, there are also important differences between transplantation and pollination. While in transplantation an autogenous condition is the most favorable one for a satisfactory union between host and graft, in fertilization identity of the specific substances which come into play is in many cases unfavorable for the production of a fertilized ovum. There is an additional difference in that in the former all degrees of gradations in the results occur, whereas, in fertilization we find either compatibility or non-compatibility, the latter leading to sterility; no inter-grades exist as far as the end result consisting in the fertilization of a single ovum by a spermatozoon is concerned. In the pollination process, itself, it is nevertheless possible to recognize certain gradations in the degree of compatibility between the male and female cells, as is indicated by the varying rapidity of the downgrowth of the pollen-tube, and in some cases, by the number of eggs which are fertilized and of embryos which develop in a normal manner.

in plants it is assumed that this is very small, but there is the possibility that, actually, also a larger number may control this mechanism. In ascidians, it is at least possible that the number of determining genes may be considerable and that in this respect the latter resemble the factors which determine the individuality differentials in the somatic cells of higher organisms.

In conclusion it may then be stated that while in transplantation of tissues in higher adult organisms incompatibilities in the interaction of host and transplant are avoided only when autogenous relations exist, in fertilization, in general, a homoiogenous relationship produces the most adequate results and the subsequent perfect development of the embryo, although in various plants and animals autogenous fertilization occurs normally, without injurious consequences. As to heterogenous fertilization, this leads, as a rule, to injurious results in plants as well as in animals, but in different instances the distance in relationship between spermatozoon and egg, which results in abnormalities, varies. Thus in the case of Zea Mays, Demerec observed that in the variety "everta", homoiogenous as well as autogenous fertilization was successful, but that fertilization with other varieties of Mays did not succeed. Usually we must assume that species differences between male and female plants produce an injurious effect greater than that characteristic of variety differences. On the other hand, certain heterogenous fertilizations in Echinoderms may lead to the formation of normal organisms as far as somatic differentiation is concerned, although the parents belong to different genera or even to different orders.

In general, homoio-fertilization is the normal process most conducive in plants and animals to an undisturbed development of the embryo and the chromosomes and cytoplasm of the germ cells are not adversely affected by such a relationship. We find, then, in the interaction between spermatozoon or pollen-tube and egg or ovary, reactions which presuppose the presence of individual and species substances or of mechanisms which are characterized by a great sensitiveness to these individual and species differences. The individuality differentials of higher adult animal organisms are not yet developed in these less developed cells and tissues, but there is reason for assuming that the latter possess the precursors of organismal differentials. It is possible that such precursor substances are involved in the individual reactions noted in these cells and tissues, at least in some instances; on the other hand, it is also possible that substances of a special kind are concerned in these reactions. Such substances and mechanisms of a special kind are present in certain protozoa, and the terms "organismal and individuality differentials" apply to these cases only in a wider sense. In a specific sense, they refer only to the adult tissues of higher vertebrates.

Inbreeding: There is a condition intermediate between self-fertilization and homoiogenous fertilization, namely, syngenesio-fertilization. This is a process which corresponds to inbreeding. In higher organisms, close inbreeding is effected by means of successive, long-continued brother and sister mating. As a result of this procedure the genetic constitution of such inbred individuals becomes gradually more and more similar, until in the end, syngenesio-

tion of hybrids, the germ cells in the latter may have abnormal numbers of chromosomes. Besides, abnormalities in the reduction division and, furthermore, a non-disjunction of chromosomes may be observed. Still later, incompatibilities may become manifest between the nuclei of the male and female germ cells if fertilization between hybrids should be attempted. Conditions are, then, in these respects, analogous in plants and in animals; in both, a *homoiogenous* relationship between the substances produced in cells belonging to the male or female organism, as well as between the cytoplasm and the nuclei of the gametes, is most conducive to the normal development of a new organism.

II. While in higher plants the prevention of self-fertilization occurs in many species, in hermaphroditic animals it has been observed so far only in several ascidians, and especially in Ciona. In other animals, for instance, the oligochaetae, self-fertilization may occur and lead to the normal development of the ovum, and even self-copulation may take place in certain species.

The fact of self-sterility in Ciona was first observed by Castle, and its mechanism has been studied especially by T. H. Morgan. According to Morgan self-sterility in this species depends on a condition in the egg membranes, which prevents the entrance of spermatozoa derived from the same individual, whereas spermatozoa from individuals which were not the bearers of the eggs were able to penetrate through the membranes. A short treatment of the egg membrane with acid makes the latter permeable also for the spermatozoa from the same individual; likewise, the use of dense sperm suspensions renders the chances of self-fertilization better. However, it has not been possible to extract substances from the eggs or sperm which noticeably influenced the results of self- or cross-fertilization. In different ascidian species, the readiness with which self-fertilization succeeds varies; it is greatest in Molgula, intermediate in Styela, and very rare in Ciona. It is the sameness in the genetic constitution of the spermatozoon and the ovum which tends to prevent fertilization and a difference in this constitution which makes possible self-fertilization. Morgan suggests that self-fertilization may become possible as a result of a mutation, which alters the genetic constitution of a spermatozoon and makes it unlike that of the egg. It is not known how many genetic factors are involved in this process of self-sterility.

It seems then that the mechanism which prevents self-fertilization in Nicotiana, Petunia and some other plants, and in ascidians, is not the same, inasmuch as in the latter it depends on the relations between spermatozoon and egg membrane, whereas, in the former, it depends largely on the interaction between ovary and pollen-tube; but also in plants, self-fertilization may be inhibited in certain instances by incompatibility between the fertilizing element in the pollen-tube and the ovum, as well as by the usual mechanisms. The processes which prevent self-fertilization in these plants and in ascidians agree, in so far as in both, genetic factors determine primarily whether or not self-fertilization can take place; but the effects exerted by these genetic factors differ. As to the number of genetic factors involved in these processes,

this strain by brother and sister mating no further improvement occurs, and very often deterioration finally takes place as a result of close inbreeding.

East and Jones explained the results of inbreeding in accordance with the rules of Mendelian heredity. The parents belonging to two different inbred strains possess genetic constituents which differ from each other. In fertilized ova, giving rise to the F_1 generation, a large number of the dominant factors from both parents are brought together, and this combination may cause an increase in size, fertility and strength of the F_1 hybrids. This is perhaps due to the fact that mutations are mostly recessive and injurious and that these injurious effects become manifest if two recessive alleles are combined in the offspring. In the F_1 generation, the chances that these injurious recessive factors become manifest are slight as compared to the genetic constitution in inbred strains. If the F_1 hybrids are inbred the advantages gained in F_1 disappear again. In the F_2 and following generations, these dominant factors again become segregated in the large majority of the individuals and a loss of the advantages gained in the F_1 generation may take place, until, as the result of continued inbreeding, the individuals have again reached a condition in which they all have acquired essentially the same, or at least a very similar genetic composition, and then no further deterioration needs to take place. The increased vigor in the F_1 generation of hybrids, due to the bringing together in the same individual of factors which are derived from different lines, and especially of dominant favorable genes which prevent the injurious effects of recessive mutants from becoming manifest, is a condition called "heterosis". In accordance with what we have stated above, inasmuch as in inbreeding in the beginning two individuals are united, belonging not to two different species but to the same species, although to two different lines or perhaps to different varieties, it would be preferable to designate this stimulated state in the F_1 hybrids as homoiosis, depending on a "homoiozygous" in contrast to an "autozygous" condition of the gene sets. Such a state of homoiosis would then, in the course of further inbreeding be followed by a state of syngeniosis and ultimately by one approaching autosis. In some instances, even a certain stimulation may result from fertilization, when the two germ cells belong to different though closely related species. In the latter case we would have to deal with a true heterosis. However, according to common usage the term "heterosis" is meant to signify the beneficial effects derived from the fact that unlike genes derived from unlike parents are combined in the same individual, and furthermore, it attributes to this unlikeness of the genes certain effects without regard as to whether the relationship of the genes is a heterogenous or a homoiogenous one. If it is desired to express merely the mutual strangeness of the genes derived from the two different parents, we might apply the term "allosis" to the condition usually designated as heterosis.

According to East and Jones, the injurious effects of inbreeding are then due to combinations of certain recessive allelomorph genes in the same individual; injurious conditions which had existed previously in a potential

fertilization may approach auto-fertilization. Thus a homozygous constitution may be nearly attained in both the germ cells as well as in the somatic cells of the adult forms, in constrast to the heterozygous condition which characterizes, as a general rule, individuals belonging to the same species but to different families or lines, which are not closely related to one another in their genetic constitution and whose germ cells unite in the process of homoio-fertilization. As the outcome of continued inbreeding, individual differences are more and more lost.

As stated, the individuals of higher species of animals are, usually, in accordance with accepted terminology, heterozygous as far as their genetic constitution is concerned. However, in order to indicate the relationship which exists between the different types of fertilization, on the one hand, and of transplantation and organismal differentials, on the other, it might be advantageous to designate as homoiozygosis the normal condition resulting from homoio-fertilization, which, as we have seen, corresponds to a homoio-transplantation. As a consequence of close inbreeding the normal homoiozygosis passes, then, into a state which might be designated as syngenesiozygosis, until at last a condition is approached corresponding to autozygosis, but generally designated as homozygosis. By adopting the term "autozygosis", we would express the genetic relationship to one another of the gene sets which have been brought together in the fertilized ovum and in the individual developing therefrom, in cases in which self-fertilization is the normal process, or a genetic relationship which may be approached in cases in which long-continued close inbreeding through many generations has preceded the mating of the germ cells. Heterozygosis, in the sense in which this term is used by geneticists, would then correspond to homoiozygosis, and the homozygous condition of the geneticist would correspond to autozygosis, in the sense in which the corresponding terms would be applied in transplantation. In the genetic analysis of the effects of inbreeding and of transplantation, we have to deal with closely related problems; but this relationship is somewhat obscured by the terminology used, and, in particular, by attributing to the terms "hetero" and "homoio" different meanings in the case of fertilization and transplantation. The term "heterozygosis", as it is used in breeding, is really meant to designate a dissimilarity in the gene sets of the different individuals which are mated. In order to accentuate the mutual strangeness of the gene sets or genes combined in zygotes, this condition might be designated as "allozygous", in contrast to the "isozygous" condition, which would correspond to the homozygous state in the ordinary meaning of this term.

In our discussion of transplantation we have analyzed the effects of close inbreeding on the fate of the graft. It may therefore be of interest to compare with the latter, the effects of close inbreeding on the character of the offspring. It has been observed that in many instances the individuals of the first generation, F_1, derived from two parents possessing genetic constitutions differing within a certain range, show an increase in size, fertility and strength, as compared with the parents, but in continuing the breeding of

limit, toxic effects would predominate and disharmonies would occur in the process of fertilization or in the development of the embryos. If we accept this mode of interpretation, we should have to assume that in homoiogenous fertilization, which represents the normal process, the injurious effects of inbreeding are avoided, because in the former case there is provided the stimulation which slightly toxic substances exert on the developing ovum. We would then have to deal with substances, the character of which depends upon the relationship between the male and female germ cells, a certain distance of relationship, but one not exceeding a definite limit, giving the best results.

Of interest in this connection are also the experiments of Demoll, who found that the injurious results of inbreeding in mice can apparently be neutralized by administration of small doses of arsenic to the breeding individuals. It seems, however, that in the deterioration caused by inbreeding we have essentially to deal with genetic reactions and that arsenic, if it should be potent at all, merely prevents some of the injurious results from becoming manifest, without essentially changing the underlying causes of the deterioration. Such a method would therefore represent merely a symptomatic treatment, and with this interpretation agrees the fact mentioned by Demoll, although not interpreted by him in this way, that following the cessation of the arsenic administration the injurious consequences of inbreeding again became manifest.

Demoll furthermore attributed the favorable effect of homoiogenous combinations of sperm and egg to the formation of antibodies, the strange spermatozoon acting as an antigen in the egg and eliciting here, or in the developing embryo, the production of antibodies, which interact with the antigen. However, the production of antibodies presupposes the presence of mechanisms which, to our knowledge, form only during the later embryonal, or even post-embryonal life. Within the same organism all the constituent normal parts have, as far as their mutual relations are concerned, an autogenous character and they are therefore not able to function as antigens. Thus it is hardly conceivable that in mechanisms so well regulated as are those of embryonal development, abnormal processes of a variable character, such as the formation of antibodies, should play a role.

We mention these physiological viewpoints, although the interpretation of East and Jones, as to the mechanisms by means of which inbreeding exerts its injurious effects, seems to have been generally accepted by geneticists. However, genetic and physiological modes of interpretation are not necessarily mutually exclusive.

state, but which had been hidden, thus become manifest. The inbreeding as such is not necessarily injurious, provided the genetic constitution of both parents is a very favorable one.

The deterioration caused by inbreeding in animals can, perhaps, to a certain extent be mitigated and delayed through continuous selection of the most vigorous individuals of the inbred strain for breeding purposes; such a selection was made in the breeding experiments in rats by H. D. King and it is possible that by these means a severe deterioration was avoided, at least for a long time. We may assume that presumably in using the strongest individuals, in most cases also the most allozygous (or heterozygous, according to the usual terminology) individuals were chosen, and thus the approach to an autozygous condition was delayed. Evidently a homoiogenous combination of genes in a zygote and in the individual subsequently formed is most favorable for the best development of a higher animal organism; conversely, a condition of autosis in fertilization may lead to deterioration. In this respect gene combinations in the fertilized ovum differ from the combinations of gene derivatives, the individuality differentials, as they are accomplished in transplantation of tissues. Here, as we have seen, the autogenous combination is the most favorable one; a syngenesio-, and still more so, a homoio- and a hetero-combination are injurious. However, it is possible also that a combination of genes derived from two unlike parents may lead to a summation of two beneficial conditions and that this summation may produce favorable physiological conditions in the hybrid. Such an effect seems to have been observed by Robbins in two races of tomatoes in which the hybrid F_1 presumably possessed the combined ability of both parents to synthesize certain vitamines B.

From a physiological point of view, it has also been suggested that a combination of genes which differ within a certain range of intensity in the F_1 generation, leads to the development in the embryo of a substance or of substances, which are slightly different from those to which the fertilized ovum and the developing embryo are adapted, and that this condition if present within a certain range of concentration exerts a stimulating effect, while a substance which exceeds a certain degree of strangeness may cause injurious effects. This formulation recalls the so-called Arndt-Schultz rule, according to which very small doses of toxic substances, instead of having an injurious effect, on the contrary, may exert a stimulating effect. It was especially Löhner who, in comparing the effects of inbreeding and of fertilization of ova by less nearly related sperm, applied the Arndt-Schultz rule to their analysis. We should then attribute the advantage of homoio-fertilization over close inbreeding to the stimulation caused by a greater mutual strangeness of the genes in the former process, as compared with the great similarity of the genes in the latter. Furthermore, cross-fertilization between different subspecies, or between certain very nearly related species, on account of the still greater mutual dissimilarities of the combining genes, might be even more beneficial and exert also the effects characteristic of heterosis (allosis); however, if the dissimilarities between the genes exceed a certain

is concerned. He made still finer gradations in accordance with Poll's terminology, distinguishing between tokonoth hybrids, which are fertile, and steironoth hybrids, which are sterile; the disharmony in the constitution of the parent strains giving rise to the former, should be less than that giving rise to the latter. Accordingly, he finds that exchange of tissues between species with tokonoth hybrids gives the better results. We shall first cite certain examples of Schultz's observations and then discuss some of the factors complicating his transplantations. Such a discussion will provide an opportunity to state also some of the principles which apply to transplantation in general.

I. *Experiments in Amphibia.* Skin of Bufo vulgaris transplanted to Bufo viridis remained alive one hundred days, and the reciprocal transplant, fifteen to thirty days; these two species can be hybridized. Skin grafted from Rana temporaria to Rana arvalis lived eighty days, the reciprocal transplant, one hundred days. In these cases in which the skin was exchanged between hybridizable species the results were therefore relatively good. On the other hand, skin transplanted from Hyla arborea to Rana esculenta remained alive only ten to twenty days. These two species not being hybridizable, the life of the transplant was shorter. However, also transplantation of skin from Rana temporaria to Rana esculenta may give very good results and the graft may remain alive for more than one hundred days, although these two species are not hybridizable. Exchange of tissues between urodele and anuran amphibia was unsuccessful.

The principal results obtained by Schultz in the heterotransplantation of amphibian skin may be summarized as follows: Length of time during which transplants remained alive after: (*a*) *Transplantation of skin between hybridizable species.*

Exchange of tissues between Rana temporaria and Rana arvalis:
70 to 105 days.

Transplantation from Bufo vulgaris to Bufo viridis: 100 days.
From Bufo viridis to Bufo vulgaris: 15 to 30 days.

(*b*) *Transplantation of skin between non-hybridizable species.*

From Bufo viridis to Rana esculenta: 10 to 20 days.
From Hyla to Rana: early death of host as well as of transplant.
From Rana esculenta to Rana temporaria: 40 days.
From Rana temporaria to Rana esculenta: 130 days.
From Salamander to Rana esculenta: 8 to 10 days.

It is evident that there is no complete correspondence between compatibility of host and transplant and hybridizability. It is furthermore probable that toxic actions, due to other factors than organismal differentials, play a role, at least in some of these transplantations.

II. *Experiments in Birds.* Schultz finds that the skin of the canary when transplanted to hybridizable species remains alive up to twenty-five days and during that time shows mitoses, whereas after transplantation to the pigeon, with which the canary is not hybridizable, the skin is found necrotic after seventeen days. Skin exchanged between pigeon and laughing dove, which are hybridizable, remains alive up to thirty days, during which time mitoses

Chapter 5

The Relations Between Hybridization and Transplantation

IN THE PRECEDING two chapters we have analyzed the relationship which connects fertilization and hybridization with the organismal differentials of the organisms which play a part in these processes, the term "organismal differentials" being used in the wider sense. We have stated that in fertilization and hybridization the interaction between the male germ cells and certain somatic tissues in the female, the interaction between the chromosomes of spermatozoon and egg, and between the genes they contain, and also the interaction between the cytoplasm of the ovum and the male and female nuclear substances, as well as the action of certain substances which develop during embryonal development, have to be taken into account. It was considered at least possible that some of the substances involved in these processes are the precursors of the individuality differentials of the adult organisms. In transplantation of adult tissues we are concerned with the relations to each other of fully developed organismal and organ differentials in host and transplant. It is certain that the results in both transplantation and hybridization depend upon the genetic relationship between the two interacting organisms. While, thus, transplantation and fertilization, and in particular hybridization, have certain important factors in common, they differ in other features, and we should therefore expect, in addition to certain parallelisms between the feasibility of hybridization and transplantation, the occurrence of definite differences between these two processes. These have been discussed from general points of view in the preceding chapters, when we analyzed and compared transplantation in higher adult and in phylogenetically and ontogenetically more primitive organisms and the relations which exist between transplantation and fertilization.

There still remains the question as to whether actual experiments in transplantation support the assumption that a parallelism exists between the ability to make successful transplantations between different species and the ability to hybridize these species. Schoene suggested, in 1912, that heterotransplantation might be possible between hybridizable species, but he also pointed out that while hybridization can take place between rat and mouse, transplantation of skin from rat to mouse, and vice versa, does not succeed; however, it is doubtful whether hybridization between rat and mouse can actually be accomplished either.

As stated previously, the most extensive experiments in which the existence of a parallelism between transplantability and hybridization was tested were carried out by W. Schultz. He attempted to prove that these two conditions follow a parallel course and that a wide cleft exists between hybridizable and non-hybridizable animals as far as the mutual transplantability of their tissues

death of the transplant. Similarly, after transplantation of cat ovary to rabbit there was early degeneration, although at first there may still have been noticeable some mitotic activity.

As we have seen, in a general way a parallelism may be expected to exist between the transplantability of tissues of certain organisms and the possibility of hybridizing them, and, on the whole, the experiments of Schultz indicate the actual existence of such a parallelism; but there are quite a number of exceptions to this rule and to some of them we have already drawn attention. Thus reciprocal hybridizations may give different results and such results may not correspond to those which are found in the case of corresponding reciprocal transplantations. We have pointed out the differences which exist in the significance of autogenous, syngenesious and homoiogenous relationships in hybridization and in transplantation. While the results of heterogenous relationships are more similar in hybridization and transplantation, a perfect correspondence is lacking even here. But a strict parallelism should not be expected, because hybridization and transplantation, as preceding chapters have shown, represent in some very important respects very dissimilar processes.

To mention some of these differences: The chromosomes of horse and donkey meet in the somatic cells of the mule without any apparent injury to cells resulting from this heterogenous combination. On the other hand, skin of the horse cannot be grafted successfully to the donkey, nor does the reciprocal transplantation succeed. Furthermore, the fact that although hybrids between two species may be well formed and strong, yet the eggs of the female hybrid may not be fertilized by the spermatozoon of a male hybrid, can be readily understood if we consider that the function of the male and female chromosomes is not the same in the germ cells and in the specialized somatic cells of the hybrids. The chromosomes of the germ cells undergo synapsis and reduction divisions, which are very complex processes. Before reduction division has taken place, the sex cells and the surrounding somatic cells have the same set of genes, but they differ following this occurrence. During reduction division in the hybrids, abnormalities may arise, which prevent the formation of healthy spermatozoa and ova and thus lead to sterility.

Schultz, in general, seems however to assume that the mutual interaction of sex cells and of somatic cells is of the same kind, and that the germ cells are more differentiated than the somatic cells, because they have the potentiality of reproducing the whole organism. But early ontogenetic stages of tissues do not yet show the same degree of differentiation of organismal differentials as do adult tissues. Likewise, there is evidence for a phylogenetic evolution of organismal differentials. Because homoio- and even heterotransplantation may succeed in certain amphibia, it does not necessarily follow that such transplantations must succeed also in mammalian organisms.

If two animals of the same species differing in certain characteristics, as for instance, in the pigmentation of certain parts of the skin, are mated, then in the F_2 generation a segregation of these allelomorphs may take place. Two individuals, A and B, belonging to the same litter, may therefore differ in the color of a certain part of their skin. Schultz holds that it should be more

are found, while in non-hybridizable forms the results are not so good. Pheasant and chicken give steironoth hybrids; skin transplanted from the former to the latter species shows mitoses after fourteen days. In skin transplanted from chicken to pigeon, which are non-hybridizable, mitoses may be found after twelve days. After transplantation of skin from the domestic to the musk duck, which are likewise non-hybridizable, necrosis is found from the eleventh day on.

Exchange of skin between hybrids of Pharaniamus and another species gives good results, the skin remaining alive for twenty-eight days, while skin transplanted from such a hybrid to one of the parents was found living after eighteen days. However, in these transplantations the exact relationship between donor and host was not definitely known; the hybrids may have been brothers and sisters and therefore Schultz may actually have carried out syngenesiotransplantations in exchanging pieces of skin between them.

III. *Experiments in Mammals.* After transplantation from rabbit to hare, which are hybridizable, skin was found preserved after thirty-five days, but grafts from a wild to a domestic rabbit were necrotic after thirty days. Skin of cat transplanted to rabbit was still alive and showed mitoses after eleven days; the beginning of necrosis was observed after fourteen days. But skin of rat transplanted to mouse and the reciprocal graft showed necrosis from the eleventh day on and there was marked lymphocytic reaction.

In case of transplantation of skin from one variety to another belonging to the same species, the results were good. Thus, after transplantation from albino to hooded rat the graft was found preserved after thirty days and showed mitoses at that time. Similarly, when skin from an albino Angora rabbit was transplanted to a French grey rabbit, the results were satisfactory.

However, the findings of Schultz, that transplants between different varieties, such as those mentioned in the case of the rat, behave exactly like ordinary homoiotransplants within the same species, do not quite agree with our own. Furthermore, Schultz (1915) assumed that no differences existed in the results of auto- and homoiotransplantation of skin, although marked differences between these two types of transplantation had already been well established.

In addition, Schultz carried out transplantations also of ovaries. Previously, he had found that within the same species (rabbits or guinea pigs) ovaries can be transplanted to males as well as to females. In the former, they remain alive for at least four months and he concluded therefore that transplantations between different sexes are less injurious than those between different species. Furthermore, the exchange of ovaries between different varieties is successful; thus, ovaries transplanted from one variety of guinea pigs to another may survive for longer than one hundred and fifty-eight days. However, regeneration of ovarian tissue takes place only after homoiotransplantation. He believes that ova, follicles, and other ovarian structures behave after transplantation in a parallel way, and assumes, therefore, that the same factors dominate the fate of the germ cells and of the surrounding ovarian tissue. After heterotransplantation the results were unfavorable, even in nearly related species; exchange of ovaries between dog and fox soon led to the

restrictions, the existence of a parallelism between the transplantability of tissues and the hybridizability of the organisms from which the tissues are derived.

A much more complete correspondenee between the effects of hybridization and transplantation was observed in the more recent experiments of von Ubisch, who used, however, not adult organisms for transplantation, but early embryonal stages of echinoderms. When he transplanted the micromeres, which give origin to the skeleton, from one species, or even from one order, into another one in which the character of the skeleton was different from that of the first species or order, he observed the formation of an intermediate skeleton in the plutei derived from these chimaerae; this intermediate condition may represent either a mosaic of the skeletons of host and donor, or a still more perfect combination. If hybrids were produced between the same orders or species which were used for grafting, the hybrids developed a skeleton which was similar to that which developed in the eorresponding chimaerae following transplantation. It may be assumed that the nuclei of the two individuals which give origin to the third individual largely determine the results in both hybridization and in the formation of chimaerae through transplantation; and although in the hybrid every nucleus contains both maternal and paternal material, while in the chimaerae some cells have only nuclei of the host and others only nuclei of the donor, still, in hybrids and chimaerae the nuclear material from both parents, or from both host and donor is present. This might explain the similarity in the results of transplantation and hybridization. However, whereas the ehimaerae eontain eytoplasm of both species, the hybrid contains only the maternal cytoplasm. Therefore the results of reciprocal hybridization may differ greatly, because the cytoplasm, which is present only in the female sex cell, differs in reciprocal crosses, whereas in the case of chimaerae, since both parents contribute cytoplasm, the cytoplasm and therefore also the results of reciprocal transplantations are the same. But this explanation may hold good only for transplantation of very early embryonal material; we have seen that in further developed organisms the results of reciprocal transplantations may differ.

In the primitive organisms employed in von Ubisch's experiments, the cells and tissues were still very plastic and they possessed the precursors of, rather than the fully developed organismal differentials, facts which may account for the fargoing parallelism found in this instance between the results of hybridization and transplantation, while such a parallelism is very much less complete in experiments in which adult tissues are used.

Concluding Remarks

From the experiments on which we have so far reported, it seems to follow that organisms in general represent organismal equilibria which in the case of the most differentiated organisms may be autogenous; this means that all the various constituent parts of organisms possess in common certain chemical characteristics, which differ from those of all, or almost all, the other organisms, and which prevent a tolerance for contacts between tissues derived from strange organisms and, instead, cause reactions of aggression or defense. This applies not only to complex metazoa, but also to certain free-living cells.

difficult to exchange skin from areas in A to B, which differ in color, than from areas where the color is the same in host and donor. According to the concept of organismal differentials, on the other hand, the same individuality differential should attach to the black and to the white skin in the same animal, and it should make no difference as far as the reaction of the host against the transplant is concerned, which part of the skin of A is grafted to B. Of course, the tissue differentials of these two parts of the skin might differ and it might thus be easier to transplant pigmented than unpigmented skin, but this difference would apply also to transplantation of white and pigmented skin in the same individual. We must assume that the tissues in the same individual possess the same organismal differentials and that these alone determine the specific reaction of a particular host against a transplant, while tissue and organ differentials would call forth the same reaction in all hosts, irrespective of the character of the organismal differentials in host and transplant. When differences in tissue or organ differentials are superimposed, in a certain individual, upon those in organismal differentials, the former do not call forth reactions specific for an individual in the same sense in which the latter do. This holds good in general, although in some cases the character of the tissues may help to determine the reaction of the host against individuality differentials.

Both Schoene and Schultz stress the importance of athrepsia in transplantation, by which is understood a condition in the graft caused by lack of foodstuffs, mainly of a protein nature, but also of salts which are specifically needed by tissues transplanted into certain hosts; furthermore, importance is attributed to anaphylactic reactions. Although in a general way both these authors regard the presence of toxic substances as a possible additional factor in determining the fate of heterotransplants, the existence of heterotoxins affecting interspecies transplantation is denied by them, because it can be observed that the margin of a transplant may be better preserved than its central parts. They assume that if a heterotoxin were active in such cases it should first show its injurious effects in the peripheral part of the graft. However, as we have seen, the better oxygen supply in the periphery as compared to the center of the graft, may overbalance and obscure the effect of specific heterotoxins.

There are still other secondary factors which have to be considered and which may explain some difficulties in transplantation: for instance, the unequal sensitiveness of different tissues to the lack of a sufficient amount of oxygen during the process of grafting, or directly following it, may play a role also in the transplantation of the fertilized ovum; differences in the structure of tissues, such as the density of the cutis, may be of some importance in skin grafting; and lastly, the different effects of hormones in different hosts and in the same hosts under varying conditions, may affect the fate of the transplanted sex organ.

On account of the difficulty in obtaining a sufficient number of suitable animals for certain transplantations, the conclusions of Schultz are based on a very limited number of experiments, but they, as well as our earlier ones, indicate that within certain limits a parallelism exists between transplantability and the phylogenetic relationship between heterogenous hosts and transplants, and the experiments of Schultz in addition suggest, with certain

Part IV Tumors and Organismal Differentials

Introduction

The Nature of Tumors

In a preceding part we have discussed the organismal differentials and, in particular, the individuality differentials of normal tissues and the reactions they call forth in the host. Under certain conditions normal tissues become transformed into cancerous or so-called malignant tissues, which possess characteristics differing in certain respects from those of normal tissues. It will be of interest to inquire whether in this cancerous transformation the individuality differentials and the organismal differentials, in general, also undergo changes; but first, we shall state briefly (1) wherein some of the differences between normal and cancerous tissues consist, and (2) what causes this transformation of normal into malignant tissue.

In cancerous tissues the growth energy is increased, at first usually in a localized area; but this increase in growth energy differs from the increase observed in embryonal tissue, in that it is not accompanied by progressive differentiation and in that often irregularities in the structure of cells and their nuclei and in cell multiplications take place. Mitoses may be abnormal, amitoses and giant-cell formation may be found. In the actively dividing cells the normal differentiation of cells and tissues may be incomplete, but there are all degrees of this incomplete differentiation. The stimulated cells frequently undergo more active movement; during these movements the normal organization of the tissues may partly be lost. The cells usually penetrate into adjoining tissues, into blood and lymph vessels, and through the circulation they may be carried to distant places and here develop in the form of metastases. Cancerous growth is a dissociated growth, in which some of the regulative factors normally controlling tissues are no longer effective. To these structural changes correspond certain chemical changes. In the carbohydrate metabolism, enzymatic splitting processes (glycolysis) may predominate over oxidative processes, especially under anaerobic, but also under aerobic conditions, and substances such as lactic acid may then be produced in excess. There may be quantitative changes in the distribution of enzymes and vitamins found in various tumors, on the one hand, and in the normal tissues from which they developed, on the other; but these alterations may vary in direction, or at least quantitatively, in different types of cancer and in different species of animals. There may be still other changes, chemical or structural; however, it is not certain whether these modifications are primary and causal, or whether they are not, rather, the consequences of the cancerous growth. Metabolic or structural abnormalities of a related kind, although

The degree to which this type of specificity between different members of the same group exists varies according to the difference in the genetic constitution of different individuals of such groups, as well as according to the phylogenetic and ontogenetic stages of development which these organisms have reached. It is very difficult, or perhaps impossible to eliminate entirely these differences between different members of the same group through long-continued, close inbreeding, which starts with two different individuals; but by these means these differences can at least be very much mitigated in the course of time, the length of which varies in the case of different species. These specificities have reached their most fargoing development in the highest organisms, which otherwise are the most rigid and the least modifiable as far as their tissue and organ constitt tions and the interrelation between the latter are concerned. They are at the lowest stage of development in the phylogenetically and ontogenetically most primitive organisms, especially as far as the manifestation of these reactions is concerned. The mechanisms which underlie such specificities under different conditions vary in different stages of the phylogenetic and ontogenetic evolution. In the course of the former, these specificities, or at least their manifestations, are newly created step by step, while in ontogenetic development they are present in the form of precursor substances and mechanisms, which in the end lead to the complete formation of systems of the individuality differentials of the higher organisms. The substances and the mechanisms on which the maintenance of these equilibria depends may accordingly vary to a certain extent under different conditions.

Thus, in some free-living single cells such specificities may exist; but the substances or mechanisms underlying them, and the reactions which reveal their existence may differ, here, in certain respects from those found in higher organisms, and such differences probably exist also in regard to the genetic constitution which determines these specificities. On the other hand, the results achieved by these various modes of interaction of different species are very similar in unicellular and the more complex organisms. As far as the individuality differential reactions are concerned, some very finely developed mechanisms, indicative of autogenous equilibria, are found in certain infusoria among the protozoa and also in some primitive plants; however, in these unicellular organisms also, environmental conditions, in addition to the genetic factors, seem to enter into the determination of these interactions to a greater extent than they do in higher animals. Moreover, in unicellular organisms there seems to be superimposed upon these autogenous equilibria, a second type of mechanism, corresponding to the fertilization process; it resembles the types of interaction which occurs between the eggs and spermatozoa in higher organisms. In the latter, the point of equilibrium is situated in the homoiogenous rather than in the autogenous zone in the spectrum of relationships. Different genetic and phenotypic mechanisms underlie these processes of interaction in transplantation and fertilization and these mechanisms may vary also in different organisms, as for instance, in ascidians and in some plants. However, there are indications that even in fertilization genetic constellations, similar to those which determine the individuality differentials, may also play a part.

change normal into cancerous tissues. Lastly, certain metazoan parasites, such as Bilharzia (Schistosoma), acting on the urinary bladder, the nematode Spiroptera neoplastica, affecting the fore-stomach of rats and mice, and Taenia crassicollis, causing sarcoma in the liver of rats, may function as cancer-producing agents.

All these stimulating factors have in common that they initiate and maintain long-continued growth processes. But hormones are the only natural physiological agents known so far, which in this way induce cancerous processes through their normal function. All others are abnormal agents. Structurally estrogenic substances may or may not be related to the carcinogenic polycyclic hydrocarbons. Under the influence of these stimulating factors the growth processes, in the tissues on which they act, become step-by-step more intense, until they end in irreversible cancerous proliferations. Somewhere during this preparatory process a state of sensitization of the tissue is reached, so that from this point on, without as yet being cancerous, the tissue will continue in its progression to cancer as a result of normal metabolic or mechanical factors and without the further aid of the specific cancer-producing agent. While the various stimuli which initiate these growth processes are important in the analysis of cancerous changes, the growth processes which they all set in motion are probably the most characteristic and the most important factor in the origin of cancer. It has been tentatively suggested by us that during this preparatory growth period, an autokatalytic growth substance is produced or increased in amount step by step and that this effects in the end the irreversible cancerous state.

(2) Hereditary genetic factors may co-operate with the stimulating factors in inducing cancer. In general, the genetic factors, as far as they relate to the development of cancer, are limited to a specific organ or tissue and the mechanism of the hereditary transmission of cancer in a certain organ may differ from that in another organ. Different tumors are, therefore, independent of one another as far as their genetic determination is concerned. These hereditary factors may be of diverse kinds; they may consist in inherited malformations of tissues or in certain diseases which cause abnormal stimulation of tissues in localized areas. In the case of mammary gland carcinoma of the mouse, which is a very common type of cancer in that species, it has been found that, in the main, differences in the hereditary tendency to mammary carcinoma in various strains corresponds to the graded ability of the mammary gland tissue to respond with growth processes to the action of ovarian hormones. These differences in the responsiveness to growth stimuli is the essential factor underlying the hereditary tendency to the development of mammary gland carcinoma, and the same factor is presumably active also in other types of cancer. In addition, a virus-like substance, transmitted to the nursing child with the milk of the mother, but present also in certain organs and in the blood, participate in the production of mammary gland carcinoma. The frequency of mammary gland carcinoma in some strains may be zero and in others 100 per cent; normally it affects, almost exclusively, females, because the hormone, estrogen, which in this

usually differing in certain respects from those noted in cancers, may be found also in other types of abnormal growth, as, for instance, in certain types of regenerative or of the so-called inflammatory growth, or even in certain instances, of excessive hormonal-correlative growth. Cancerous tissue behaves essentially like an originally normal tissue stimulated to grow and to move under more and more abnormal conditions.

However, essentially cancerous growth differs from other types of abnormal growth in that it is an irreversible process, whereas regenerative, hormonal and inflammatory growth ceases whenever the causes which induced these proliferations are removed. Once a normal cell has become cancerous, it may die, but as far as is known at the present time, it will not return to the normal state. All kinds of tissues which have the ability to grow may become cancerous; cancerous epithelial tissue is called carcinoma and cancerous mesenchymatous tissue is called sarcoma. Also, embryonal tissues may become cancerous and parthenogenetically developing eggs may give origin to teratomas, in which many varieties of tissues may be represented. Cancerous growths developing from tissues of the adult organism are classified in accordance with the character of the tissue from which they are derived. In addition to the fully cancerous, malignant tissues, there exist others which are in a transitional state. They form the so-called benign tumors, in which the growth is increased and abnormal but slower than in the typical cancers, and in which it takes place not by infiltration of the neighboring tissues but by concentric extension, the differentiation of the affected tissues usually occurring in a more normal manner than it does in cancerous tissues. However, all kinds of gradations exist between normal tissue, benign tumors and cancer, and while in the majority of cases a benign tumor remains benign throughout the life of the individual, it may change into a malignant one. When cancer particles are transplanted into other animals of the same species, they may, in the new host, maintain their malignant growth or they may become necrotic, and are then absorbed. The readiness with which different tumors can be transplanted into different hosts and individuals differs greatly.

As to the factors which induce this transformation of normal into cancerous tissue, three main sets of conditions can be recognized: (1) a stimulation of growth which usually extends over long periods of time and may show various degrees of intensity, (2) genetic factors, and (3) viruses or virus-like substances. 1. *Stimulating factors:* Hormones may function as stimulators of the cancerous transformation and they elicit cancerous growth in those tissues in which they induce, also, under normal conditions, growth processes. Then there are special chemical, so-called carcinogenic substances, tar and some substances which are constituents of tar or related compounds; they are very efficient in causing cancer, but are not as selective in regard to the tissues which they affect as are hormones. Dibenzanthracene, benzpyrene and methylcholanthrene are the best known among these substances. Also, various injuries, inducing long-continued, regenerative growth, may have similar effects. Ultraviolet light, X-rays and radium may in the end,

of the cancerous process, although we cannot rule out the possibility that a virus or virus-like substance may be a hidden cause of all cancers.

Given these fargoing changes in the reactions which normal tissues undergo in becoming cancerous, the problem arises whether these remarkable changes at the same time induce alterations in the character of the individuality differentials of the tissues or in the reactions of the host against the abnormal transplants. To answer these questions, we present in the following chapters the data which are relevant in this regard.

instance represents the stimulating factor, is present mainly in the female sex. It has furthermore been observed in this kind of cancer that in hybrids the mother strain is much more potent than the father strain in determining the cancer rate, and this is due to the factor transmitted with the milk from the nursing mother to the offspring. An inverse relation seems to exist between the intensity of stimulating factors and the strength of the genetic factors needed for the transformation of normal tissues into cancer. Either of these two sets of factors alone may be effective in certain cases, if it reaches a given intensity. This relation can be expressed by the equation:

H (hereditary constitution) $\times$ S (stimulation) = C (cancer).

(3) In the main, three kinds of viruses or virus-like substances are known as causative factors in cancer, namely, (a) the virus of cottontail rabbits, which may give origin to papilloma, but under certain conditions, also to epidermal cancer. It probably acts as a stimulating factor, comparable to the other stimulating factors already mentioned. (b) The milk factor, which participates in the origin of mammary gland carcinoma in mice and acts in association with hormonal and genetic factors. It may have also a slight effect in mouse leukemia. Its mode of action is not definitely known, but there is some indication that it also may act as a sensitizing factor. (c) In avian sarcomas, agents can be separated from the tumor cells by filtration and certain other means. These agents seem to cause this type of cancer directly, without inducing first a preparatory growth period. They are largely but not entirely species- and organ-specific. They represent a carcinogenic substance in the strict sense, while the other factors apparently are growth promoters. A similar agent is, perhaps, present in renal carcinoma of the frog.

It is believed by some investigators that somatic mutations in tissue cells may be responsible for the origin of cancer; however, there are a number of facts which make this interpretation very improbable. On the other hand, mutations which affect the germ cells may determine the degree of hereditary tendency to the development of a certain type of cancer in individuals, strains or species. Three theories concerning the origin of cancer are under consideration at the present time: (1) The somatic mutation theory assumes that all the other factors mentioned cause cancer by inducing changes in the genes of a certain cell, which then becomes cancerous and gives origin to the other cancer cells. There are very serious objections to this theory, which is, therefore, in all probability not correct. (2) The virus theory assumes that viruses are the essential causes of all cancers, and that all other factors are effective only if they make it possible for viruses to invade cells and to activate their growth. While the possibility of such a function of viruses is indicated, especially in the case of avian sarcoma, there are some difficulties also to the application of this theory in many other cancerous states. (3) The theory that step-by-step increases in growth momentum of tissues lead to intermediate stages of sensitization, and ultimately to irreversible, cancerous proliferation, perhaps through the mediation of an autokatalytic growth substance. This seems at present the most likely general explanation

method of measuring the individuality differentials; what is measured in this way is not necessarily the degree of similarity or dissimilarity of the individuality differentials of host and transplant, but the ability or lack of ability of the tumor to overcome a limiting factor for the growth of the transplanted tumor tissue in a certain host. It may be regarded, therefore, as doubtful whether the data obtained in tumor transplantation can be directly applied to the analysis of the individuality differentials of tumors; notwithstanding these difficulties, there is much evidence that the organismal, and in particular, the individuality differentials, are essentially the same in normal tissues and in tumor tissues, and that the specific characteristics of cancer tissues, which differentiate them from normal tissues, are not so much due to changes in the organismal differentials as to certain other conditions.

As has been said, in tumor transplantation the main concern is to determine whether or not a transplanted piece of tumor shows continued growth, and it is customary to record the percentage of successful transplantations, of "takes," as they are obtained under various circumstances. However, besides the transplantability there are two other variable factors which should be considered in evaluating the result of transplantation, namely, (1) the growth energy of a tumor, by which is meant the rapidity of its growth, and (2) the latent period intervening between the time of transplantation and the first definite manifestation of an expansive growth of the grafted piece. These data are obtained by measuring at certain periods the diameters of the tumor, or better still, by determining, in addition, its weight at the conclusion of the experiment. In some of our early transplantations, we gave attention, also, to these last named factors. But as mentioned, only the percentage of "takes" was recorded by the majority of investigators, and the lack of fineness of this test was not felt as a serious difficulty, especially in the earlier period of tumor research in which the peculiar properties of tumors were analyzed largely by means of transplantation. As a rule, the growth of transplanted tumors was considered as something distinct from the growth of various normal tissues. Only gradually, step by step, was the great similarity in the behavior of normal and tumor tissues after transplantation established, and at the same time the factors which differentiate tumor and tissue growth were analyzed.

The first successful transplantations of tumors in animals were carried out by Hanau, Morau, Velich, Eiselsberg and Firket. They used for this purpose, carcinoma of rat and mouse as well as sarcoma of rat. These experiments established the fact that certain tumors can be transplanted to other animals of the same species, at least for a limited number of generations. Incidentally also, some interesting observations concerning the factors on which transplantation depended were made, especially by Morau in his experiments with carcinoma of the mammary gland in the mouse. A new motive was introduced into the experimental study of tumors in the beginning of this century, in a series of consecutive transplantations of sarcoma of the thyroid gland of rats by the writer (1901) and of a mammary gland adenocarcinoma of the mouse by Jensen (1902). In these experiments, which

Chapter I

A Comparison between the Transplantation of Tumors and of Normal Tissues

WE HAVE analyzed by means of transplantation the organismal and organ differentials of the normal tissues, and we shall now proceed to the study of the corresponding differentials in tumors, which latter differ in their behavior, after transplantation, in certain respects from normal tissues. In the introductory statement we have discussed the essential characteristics of tumors, especially of cancers, and wherein they differ from normal tissues. We then inquired into the factors which caused the transformation of normal into cancerous tissues. We shall now study the various types of transplantation of tumors and compare the essential results with those obtained after the grafting of normal tissues; there are some very marked similarities, as well as some differences. In tumors, we shall find certain complications which did not occur in normal tissues, such as an increased growth energy, which to some extent may overcome the antagonistic reactions of the host to the transplant; also, there are indications that the tumor cells themselves can undergo changes of an adaptive character during the course of transplantation, and that, in particular, they may acquire resistance to certain injurious conditions to which they are exposed in the new hosts. On the other hand, cancerous tissues may call forth in the new hosts, states of immunity or allergic reactions, which tend to injure the transplant; but there are strong indications that against these the cancer tissue may find protection to some extent, by its ability to absorb and to neutralize substances antagonistic to its growth. There exist in addition, the same problems which we had to face also in normal tissues, namely, that of distinguishing between the presence or lack of the various organismal differentials in the tumor cells, and of the manifestation of these differentials, which may depend on the rapidity of the production and discharge of the organismal differentials by the transplanted tumors or host, and on the power of resistance of the tumor cells to the injurious effects of the host. There is still a further complication: while in normal tissues we can readily follow the reactions of the host against the transplant and, with certain precautions, use these reactions as a standard with which to gauge the differences in the organismal differentials between host and graft, in the case of tumor transplants such an analysis is very difficult on account of the relatively rapid growth of the tumor tissue. Thus the finer reactions, which we used as indicators in the analysis of the organismal differentials, and especially of the individuality differentials, in normal tissues, cannot very well be used in tumors, at least, not in many cases. Instead, most investigators employ as the standard, the growth or lack of growth of the grafted tumors. This is a less finely graded

originated in a white American mouse, and which we used in many of our experiments, could be transplanted into the large majority of American white mice, but into a much smaller percentage of German or English mice. We find, therefore, all kinds of transitions between transplantable and non-transplantable tumors. The larger the number of animals which are tested for their suitability as hosts, the greater becomes the chance that in the end we shall find an animal in a mixed strain in which the tumor will take; which means that the cells remaining alive after transplantation will continue to multiply. And between this condition of relative non-transplantability and a perfect transplantability in 100 per cent of all animals of the same species, we find all intermediate grades. However, the more closely inbred a strain is, in which a tumor originated, the larger becomes the number of animals belonging to this strain into which, as a rule, the tumor can be successfully transplanted, whereas, the tumor may not grow after transplantation into other strains.

There are, in a general way, two factors which determine the degree of transplantability of a tumor, as expressed by the average number of takes, namely, (1) the relation of the individuality differential of the host to that of the transplant, and (2) certain factors which differentiate normal tissues from tumor tissues, and which may vary quantitatively in the case of different tumors; among these are variations in growth energy and processes of adaptation, which may take place between tumor and host. In all cases the individuality differentials in host and transplant seem to assert themselves, even in those tumors in which the transplantability is 100 per cent, for here, also, a transplanted tumor differs in its relation to the host from a spontaneous tumor developing in the same animal. We had already noted, in our earlier transplantations, this difference between spontaneous autogenous tumors and transplanted homoiogenous tumors. While spontaneous tumors have a tendency to recur after extirpation, transplanted tumors are, as a rule, more sharply separated from the host tissue and can much more readily be completely removed; they behave like strange organisms implanted in the host, from which they draw their nourishment but from which they often remain separated by a capsule; their vascularization is less adequate, and not rarely they grow even more rapidly in the host than do spontaneous autogenous tumors. Notwithstanding such a rapid growth of the homoiogenous tumors, it is, after all, a precarious existence which they lead in the strange host, as shown by the fact that they are usually more readily damaged by the injection of certain unsuitable substances into the host than are autogenous spontaneous tumors. These various differences in the behavior of spontaneous and homoiogenous transplanted tumors are, perhaps, partly due to the process of transplantation as such, but they are largely caused by the difference in individuality differentials of host and transplant; an injurious reaction against the transplant takes place in the strange host, and such an injurious effect is the more evident the greater the dissimilarity between organismal differentials of host and transplant. In a general way, it may be stated that these primary reactions are similar to those which are

extended through many more generations than previous ones, transplantation was used as a method for analyzing the characteristics of the tumor cells and the interaction between tumors and hosts; there was thus initiated the subsequent large number of investigations into the biology and causes of cancer, which has continued with increasing intensity until the present day and which has contributed much to the solution of these problems.

The objective of the writer was the study of the characteristics of tumor cells, of the factors which made them behave in their own peculiar way, of the possibility of separating a living agent responsible for the tumor growth from the transplanted cells; in addition, there was the analysis of the causes and mechanism of the transformation of normal into tumor cells, and, above all, the comparison between the behavior of transplanted normal and cancerous cells, which made possible a critical examination of what we now call the organismal differentials of tumors. Jensen approached these investigations primarily from the point of view of bacteriology and immunology, his central problem being the possibility of obtaining an active and passive immunity against tumor growth, similar to that which can be obtained against bacteria and their toxins. In a similar way, the subsequent investigations of Ehrlich and Apolant, Gaylord and Clowes, Bashford and Murray and their collaborators, and many other well known workers, were largely concerned with the problem of immunity, but gradually these studies have led back again to a comparison between the behavior of normal and tumor tissues, since it became more and more evident that some of the most important characteristics of tumor cells are shared with normal cells. Thus, in the end both these series of investigations contributed also to the analysis of organismal differentials in general.

We shall now compare the various types of transplantation of tumors with those of normal tissues and determine wherein they resemble each other and wherein they differ.

Auto- and Homoiotransplantation of Tumors

We have seen that normal tissues behave very differently after auto- and after homoiotransplantation. In the former, the individuality differentials of host and transplant are identical, while in the latter they are different. One of the marked differences between normal tissues and tumors consists in the fact that some tumors can be homoiotransplanted from generation to generation into a percentage of animals of the same species, which varies in the case of different tumors, whereas, such a serial homoiogenous transplantation does not succeed with normal tissues. But this is not true of the majority of tumors; while there are some which can be readily transplanted into animals belonging to the same species, irrespective of family or strain, the large majority grow only in animals belonging to the same closely inbred strain, and very much less or not at all in other strains; again, others grow in a certain percentage of mice from mixed strains of the same country in which they had developed, but do not grow in strains bred in distant countries. To cite an example: a carcinoma of the mammary gland which had

of certain tissues. We concluded further that when the intrinsic factor, which represents the essential stimulus to tumor growth, is very strong, then the substances which determine to what extent tumor cells are able to live in other individuals—the individuality differentials—may become less important in determining the fate of the transplanted tumor. However, there is a limit as to the differences between the individuality differentials of host and transplant if the intrinsic growth stimuli shall be able to assert themselves. This would represent a special instance of the more general rule that the action of efficient growth stimuli, or expressed differently, a strong growth momentum, may make it possible for tissues to overcome conditions which are unfavorable, not only to the growth but also to the life of these tissues.

Subsequent experiments of others have confirmed these observations and conclusions. Thus Borrel and Petit, Ribbert and Mann, obtained similar results in horse, dog and cat, respectively, and Tyzzer, Apolant and Haaland found the same differences between auto- and homoiotransplantation in mammary carcinoma of the mouse. While only a relatively small number of spontaneous mouse carcinomata can be readily homoiotransplanted, autotransplantation almost always succeeds. In accordance with these conceptions also, were the subsequent findings of Haaland (1910) that inoculation of a transplantable tumor in a mouse did not prevent the later development of a spontaneous tumor in this animal; nor did the growth of the transplantable tumor affect metastasis formation or a subsequent autotransplantation of a spontaneous autogenous tumor. Conversely, Haaland observed that the presence of a spontaneous tumor did not noticeably influence the take or the secondary retrogression of a transplantable tumor. Bashford interpreted these differences between the behavior of the transplantable tumors and of spontaneous tumors as an indication that the conditions of transplantation differ from those which determine the origin of a spontaneous tumor; he did not attribute them to differences in the individuality differentials of host and transplant. While it is true that the conditions determining the first origin of a tumor and its transplantability are different, the essential factor is that a spontaneous tumor represents an autogenous tissue, possessing essentially the same individuality differential as the other tissues of the indvidual in which the tumor originated, whereas the tumor transplanted into another individual of the same species represents a homoiogenous tissue with an individuality differential which differs to a greater or lesser degree from that of the host.

While the growth of a homoiotransplanted tumor does not need to affect the autotransplantation of a spontaneous tumor, there are some observations which indicate that a spontaneous (autogenous) tumor may, under certain conditions, influence the growth of a homoiotransplanted tumor. Thus it seems that spontaneous mouse tumors, which, as we have seen, in the majority of cases are very difficult to transplant into other individuals, can apparently be more readily homoiotransplanted when the host is also the bearer of a spontaneous tumor (Loeb, 1907; and Loeb and Fleisher, 1913

noted in the case of normal homoiotransplanted tissues; but added to these primary reactions are secondary immune reactions, which are much less evident in the case of normal tissues than of tumors. However, at the time when these observations were made, very closely inbred strains approaching homozygosity were not yet available; if transplantations are carried out in such almost homozygous strains, the differences in the individuality differentials between host and transplant may be much reduced, or almost entirely eliminated, and if there are still some differences to be found in the behavior of spontaneous and transplanted tumors under these conditions, these must essentially be due to changes which took place in the tumor cells in the course of transplantation. Also, normal tissues grow very much better after transplantation into individuals belonging to the same inbred strain than into those belonging to different strains, and this fact again proves the similarity of the role of the individuality differentials in the behavior of tumors and of normal tissues after transplantation.

We see, then, that even when tumors grow well in homoiogenous animals, differences which exist in the constitution of analogous tissues in different individual hosts assert themselves; but this fact was appreciated only after it had been shown that the relative readiness with which auto- and homoiotransplantation can be carried out in the case of tumors is the same as in the case of normal tissues. The first systematic investigations concerning such differences in the behavior of tumors after auto- and homoiotransplantation were made in 1901 and 1902, when we studied for this purpose a mammary adenoma of the rat, and subsequently, with S. Leopold, a mixed tumor of the breast in a dog. After autotransplantation the tumors—their epithelial as well as their connective tissue constituents—remained alive, while after homoiotransplantation they died. As to the rate of growth, the autotransplants showed the slow rate of the original tumors; but if, under the influence of pregnancy, the original tumor grew more rapidly, the autotransplants likewise assumed a rapid growth, which ceased after the conclusion of pregnancy. We drew, then, the conclusion that the composition of the bodyfluids in the individual in which the tumor originated differs in some respects from that in other individuals of the same species, and that in the former it is much more favorable for the life and growth of transplanted cells. This conception we have applied to tissue transplantation in general and as far as this conception holds good we have considered tumor transplantation merely as a special kind of tissue transplantation. We would now attribute these individual differences in the composition of the bodyfluids to the primary differences in the individuality differentials which are present in the cells of these animals, and these cellular differences are associated with secondary differences in the constitution of the bodyfluids. From such individual specific substances we distinguished growth substances of an intrinsic character, inherent in the tumor cells, and representing the essential stimulus to tumor growth, and lastly, extraneous growth substances, especially certain hormones, such as those given off by ovarian structures, and other similar substances, which were able to influence tumor growth as well as the growth

the host possessing in all probability the same or almost the same individuality differential.

The most probable interpretation of this experiment seems to be that the growth of the first homoiogenous tumor causes the production of an immune substance, injurious to the growth of this tumor. However, this injurious substance is, to a large extent, absorbed and neutralized by the growing homoiogenous tumor itself. If now this tumor is extirpated, the immune substance is no longer neutralized and it is thus able to prevent the growth of a second homoiogenous tumor. Besides, this immune body must carry a differential able to combine with the homoiogenous differential of the transplanted tumors, while the tissues of the host animal, as well as those composing the autogenous tumor, being the bearers of an autogenous differential, are not able to remove and to neutralize this substance. Substances which carry a homoiogenous individuality differential may then induce in the host immune reactions antagonistic to the growth of homoiogenous tumors, but they are not absorbed and neutralized by autogenous tissues. It would be of interest to determine whether the immunity procured in the Uhlenhuth phenomenon is a specific one, directed only against a certain homoiogenous tumor, or whether it also protects against other types which carry different homoiogenous individuality differentials. In accordance with this interpretation it may then be concluded that antibodies are produced by growing homoiogenous, but not by autogenous tumors, and furthermore, that such antibodies are neutralized by homoiogenous but not by autogenous tissues; but at any given time, the amount of such antibodies circulating in the bodyfluids may be too small for direct demonstration by the ordinary serological methods.

There is another set of experimental data which confirms and further extends these conclusions as to the importance to be attached to the difference between autogenous and homoiogenous differentials. It has been found possible (Schoene, Bashford) to immunize mice, although only to a limited extent, against the growth of a homoiogenous, transplantable mammary carcinoma by a previous inoculation of normal tissues, such as erythrocytes, embryonal material, liver and spleen. As a matter of routine, the tissue used for immunization was taken from other animals of the same species. Woglom however, tested the immunizing power of a piece of the animal's own spleen. At first he believed that the inoculations of such autogenous tissue also produced a positive result, but the subsequent experiments of Apolant and Marks, as well as of Woglom himself, showed that neither the animal's own spleen tissue nor its erythrocytes had any demonstrable immunizing action, while inoculation of the tissue of other animals of the same species was effective. We may then state that a difference in individuality differentials is a prerequisite for the production of immunity, that it is presumably the strange differential itself which is concerned in this process of immunization, and that identity of organismal differentials, in the immunizing material and in the animal which is to be immunized, precludes an effective immunization; this observation is in harmony with the fact that a transplant

and 1916). A similar but more casual observation has been made also by Apolant. In recent experiments from our laboratory, Blumenthal confirmed this difference between the transplantability of spontaneous tumors into normal mice of different strains and into mice which are bearers of other spontaneous tumors. However, this condition was found only if the hosts were below the age of 12 months, while in older mice, the growth of a spontaneous tumor did not enhance the result of homoiotransplantation.

The importance of the relations between the individuality differentials of host and tumor was quite definite in experiments in which we compared the effects of the extirpation of a spontaneous autogenous and of a homoiotransplanted tumor on the growth of a second homoiogenus tumor. In this connection we must first refer to the important experiment of Uhlenhuth, Haendel and Steffenhagen, who found that if they inoculated a transplantable rat tumor into a rat and the transplant took, it was possible to inoculate the same rat successfully with a second homoiogenous tumor. But if previous to the second inoculation the first homoiogenous tumor had been extirpated, the animal was immune and the second inoculation was unsuccessful. However, if the extirpation of the first tumor had been incomplete and the part which had been left behind grew again, then a second inoculation with the homoiogenous tumor was successful. These observations were controverted by some investigators; especially by Russell, and also by Woglom. The effect of the extirpation of the first tumor, if present at all, was attributed by these authors to the non-specific effects of the operation, and the immunity following the extirpation of the first tumor was accordingly designated as an "operative immunity". But, the experiments of Fleisher and the writer showed that the observations of Uhlenhuth and his collaborators were essentially correct, at least as far as certain types of tumors are concerned, among which may be included our transplantable mouse carcinoma IX. We found that extirpation of this tumor, when growing in a homoiogenous mouse, prevented the successful second inoculation with this tumor. Evidently the growth of the first tumor had produced an immunity, which became noticeable only after the first tumor had been removed. Furthermore, it could be shown that if pieces of carcinoma IX were transplanted, not into a normal mouse but into a mouse which, in addition to a first inoculated tumor, was also the bearer of an autogenous spontaneous tumor, the Uhlenhuth effect was also readily demonstrated as far as the influence of the transplanted tumor was concerned; but if we extirpated instead of the first homoiogenous tumor, the autogenous spontaneous tumor, no immunity was conferred on the mouse against a second inoculation with homoiogenous mouse carcinoma IX. This proves that the immunity conferred by the extirpation of the first tumor is not a non-specific "operative immunity", but must be due to a specific relation between the individuality differentials of the growing tumor and of the host. The individuality differential of the transplant differs from that of the host and of the spontaneous autogenous tumor, the autogenous tumor and the normal tissues of

However, according to this investigator the serum of rats immunized against a rat tumor does not affect autogenous macrophages of the immunized animal or those of another immunized rat, although it may injure macrophages of the rat spleen, which are homoiogenous in nature.

The analysis of the individuality differentials will be continued in the next chapters, where we shall further discuss immunity against tumors and hereditary factors as they apply to tumor transplantation.

Transplantation of Heterogenous Tumors and the Species Differential

In heterotransplantation of normal tissues we observed not only survival of the transplants, but also growth phenomena in some of the tissues, but both of these processes had a very limited duration, and growth, if it took place, was much weaker than after homoiotransplantation of the corresponding tissues; furthermore, the proliferative processes ceased sometime previous to the death of the grafts. We also noted that different kinds of tissues showed different degrees of resistance to the injurious action of the primary, preformed heterotoxins. While more sensitive tissues, such as thyroid, kidney, and also skin, were destroyed so rapidly that a pronounced cellular (lymphocytic) reaction on the part of the host tissue against the transplant could not develop, or was much diminished in intensity, heterotransplanted cartilage proved more resistant, and it lived long enough to allow a very marked connective-tissue reaction as well as an accumulation of polymorphonuclear leucocytes and lymphocytes around the graft.

If instead of using normal tissues, we carry out heterotransplantation of tumors, the results are in principle the same, although there exist some quantitative differences, which are due at least partly to the greater proliferative momentum inherent in tumors. In addition, the possibility must be considered that tumors manifest a greater power of adaptation to certain injurious conditions than normal tissues, and, as we have seen, they may be able to neutralize, in some way, substances which tend to inhibit their growth.

Under these circumstances, it is to be expected that the range of conditions under which tumors can grow should be somewhat wider than that of normal tissues after homoiotransplantation as well as after heterotransplantation, though as a rule, tumors are about as sensitive to heterotoxins as are normal tissues. After heterotransplantation of tumors there may be a preliminary period during which the growth may be quite active; but soon it ceases, degenerative processes set in, and the tumors are destroyed. The degree of growth and the duration of this preliminary period depend upon the inherent proliferative momentum of the tumor, the sensitiveness of the tissues of which it is composed, and the degree of difference between the species differentials of host and of transplant. Only if the species of donor and host are very nearly related may the growth be more intense and the growth period of greater duration. Thus heterotransplantations between rats and mice may succeed relatively well, temporarily; but the results are much more unfavorable if less nearly related species are used. Also, in the case

possessing the same organismal differential as the host, does not elicit an antagonistic reaction; it does not act as a stimulus.

There are on record, however, some observations which apparently do not agree with these conclusions. (1) According to Murphy, it is possible to influence the growth of transplanted homoiogenous or heterogenous tumors through the application of a method which affects the number and activity of lymphocytes of the host; an increase of the latter is believed to initiate or to intensify the mechanism of defense against the transplanted piece of tumor, while a diminution in the number of lymphocytes makes possible the growth of tumors, which otherwise would not have occurred. But of special interest is the additional finding that the change in lymphocytes is effective not only against homoio- and heterotumors, but also against pieces of autotransplanted tumors. An experimentally produced increase in the number of lymphocytes was found to diminish markedly the ability of the autotransplanted tumors to grow and to develop. However, in this case we may have to deal with a non-specific effect exerted on the tumor cells by lymphocytes without the intervention of organismal differentials. (2) Fibiger and Miller, in the course of experiments, in which they produced carcinomas through often repeated applications of tar to the skin of mice, found in a certain number of instances that metastases of these cutaneous cancers took place spontaneously in the lung and elsewhere. Now if these animals, during the period when the tar was applied, were inoculated several times with mouse embryo-skin, the number of metastases was thereby diminished. There would then be involved, in these experiments, apparently an effect of homoiogenous material on autogenous tissue. If these observations should be correct, we would have to assume that also in this case we had to deal with conditions of a non-specific nature, which affected unfavorably the growth of the transplant. In this connection we may recall the more recent findings of Murphy and Sturm, who showed that in embryo-skin substances are present which inhibit tumor growth and may cause the regression even of spontaneous tumors. (3) Lumsden found that when he made repeated injections of the euglobulins from the serum of sheep, which had been immunized against either human, rat or mouse tumors, into or around a spontaneous mouse tumor and then extirpated the tumor and autotransplanted a part of it, the autotransplants did not grow in the large majority of cases, although homoiotransplantation of these tumors into other mice would succeed; furthermore, as a rule the tumor did not recur after excision. He attributed this result to the development of an immunity against its own tumor in the mouse, and believed that this immunity was due to the absorption of tumor material. But, this type of imunity has apparently not yet been tested by inoculating one of the transplantable tumors in such a mouse. In accordance with such an interpretation, it would be necessary to believe that there is present in the autogenous tumor, in addition to the organismal differentials, still another constituent which calls forth this reaction. This constituent might be an organ differential or it might be a specific stimulus to tumor growth, in the latter case an "antimalignancy" constituent, in the sense of Lumsden.

tiveness to these substances, or a greater power to absorb and neutralize them was responsible for the increased resistance. That these tar cancer cells, however, still possessed the species differential of the mouse is indicated by the fact that it was possible to elicit an active immunity against them by a previous immunization of a rat with mouse liver serving as an antigen. But the best known experiments of this kind are those of Putnoky, who has been able to propagate an Ehrlich mouse carcinoma continuously in rats since 1929 by re-transplanting it every ten days. This mouse tumor grew very rapidly in rats for from ten to fourteen days, during which time many rats were killed by its growth. Following this period, necrosis and death of the tumor set in in animals which survived. In other series of heterotransplantations, various investigators have succeeded in keeping mouse tumors alive in rats and in inducing temporary growth, but invariably the growth energy has decreased after a number of successive rat to rat transplantations and then the tumors died out, although after re-transplantation to the mouse, if the tumor had not been too seriously injured, it could be propagated indefinitely.

Why is it that in these few instances it has been possible to propagate indefinitely, under the conditions mentioned, the mouse tumor in rats? Presumably the various factors enumerated above were responsible for its successful development in heterogenous hosts. The tumor chosen by Putnoky, being a very rapidly growing one, the growth momentum of the cells could overcome injurious conditions up to a certain limit. This marked growth intensity found a morphological expression in the small amount of stroma present in this carcinoma. It was to be expected that such a rapidly growing tumor would be able to absorb and neutralize a larger quantity of either natural or immune heterotoxins than would a slowly growing tumor. Additional factors to be considered in these heterogenous tumor growths are the greater power of resistance of the tumor cells and, furthermore, the strain of rats into which the transplantations are made. Putnoky found that the constant propagation of the Ehrlich mouse carcinoma in rats succeeded only if a Hungarian strain of rats was used; in English rats the tumor regressed spontaneously after about ten or fourteen days. This explains why several other investigators, who used other than Hungarian strains of rats, were unable to obtain equally favorable results. However, more recently de Baloghi succeeded in repeating Putnoky's experiments with another strain of Ehrlich mouse carcinoma. This tumor behaved biologically and structurally in a similar way to Putnoky's tumor; but de Baloghi obtained long-continued heterogenous growth in diverse races of white and gray rats. The favorable results noted in these experiments were therefore not primarily due to racial factors in the host, but apparently to the selection of the most vigorous tumors possessing a great growth momentum.

There remains the question as to whether the differences in the growth of different mouse tumors in the rat are due to a change in the genetic constitution of these tumors, implying a difference in their organismal (species) differentials. There is no definite reason for assuming such a change. These rat tumors can readily be re-transplanted into mice, where they grow in the

of normal tissues we found that the degree of species-relationship between host and donor may influence to some extent the result of heterotransplantation. Furthermore, especially those tumors which grow rapidly after homoiotransplantation may be expected to grow actively also after heterotransplantation; therefore, a rapidly growing sarcoma may be especially well suited for heterotransplantation. There may perhaps be in addition some special conditions which may enable cancer tissues to overcome more readily than normal tissues the unfavorable effects of heterogenous transplantation.

However, in general, the injurious effects which take place in strange species are cumulative and lead gradually to the death of the transplant. By re-transplanting the tumor into an individual belonging to the species in which it originated, such a cumulative action may be prevented, or at least delayed, and the tumor given a chance to recover to some extent from the injury received by the heterotoxins. After such a recovery has taken place, it may be possible to transplant the tumor again into the strange species, and this process may be repeated a number of times. Such a procedure was used by Ehrlich in heterotransplantation of mouse tumor to rat and he called it zigzag transplantation. The conditions are here, to a certain extent, comparable to those prevailing after injuring the tumor by exposure to graded degrees of heat; then, also, a recovery may take place after transplantation into a new host. But there are indications that even after transplantation into relatively nearly related species a gradual and slowly cumulative injury does, as a rule, take place eventually; while after transplantation into more distantly related species, as for instance, from mouse to guinea pig, a repeated heterotransplantation, with intermediate recovery periods, in the original species would in all probability be impossible.

However, it is not only the cumulative action of the preformed heterotoxins which prevents the continued growth of a tumor in a strange species, but in addition, it is the active immunity developing in the host against the heterogenous differential which helps to injure and destroy the transplant. Such an immunity against heterogenous tissues can be elicited much more readily and effectively than against homoiogenous tissues, and when it is well established, the critical period sets in. It might therefore be expected that if the injurious action of these immune heterotoxins were avoided by re-transplantation of the heterogenous tissue into a new, not yet immunized individual of the strange species, the results might be improved, provided we had to deal with relatively resistant and rapidly growing tumors. Such a condition apparently has been realized in more recent experiments by Ito in transplantation into rats of a squamous cell tar carcinoma experimentally produced in a mouse. This mouse carcinoma could be transplanted for many generations into rats if a re-transplantation into a new host was carried out every five or six days. But if the transplant was allowed to remain as long as ten days in the heterogenous host, it became entirely necrotic. It must be assumed either that an increased growth momentum, acquired during their transformation into cancer, made it possible for these cells to overcome the injurious action of the natural heterotoxins better than normal tissues, or that a diminished sensi-

depend upon the genetic constitution, may perhaps have been slightly modified, and that such a modification may have facilitated the growth of the mouse tumor in the rat; but we do not possess any definite knowledge as to wherein such adaptive processes consist.

There can be little doubt that even under the most favorable conditions a heterotransplantation of a mouse tumor into rats, which would lead to a permanent growth in the latter, cannot be accomplished. There remains a difference between the growth of these tumors in the rat and in the mouse, and there also remains a difference between the growth of rat-adapted mouse tumors and real rat tumors in the rat. It is therefore not possible to conclude that a heterotransplantation has been fully successful in these experiments. Furthermore, we must not identify mouse-to-rat transplantation with heterotransplantation in general. There can be no doubt that transplantation of mouse tumors into the subcutaneous tissue or into the peritoneal cavity of farther distant species would have a much more unfavorable outcome and that such tumors would undergo rapid necrosis.

While the data concerning the degree to which transplantation of tumors from mouse to rat is possible may be considered as well established, and while these data are not in conflict with the conclusion that the concept of organismal differentials applies also to the transplantation of tumors, there have been recorded, from time to time, observations which make it appear that cancers can be successfully transferred also into widely distant species. If this were a fact, it would be contradictory to what is known about the significance of organismal differentials in determining the fate of transplants. Thus it has been stated that human tumors can be transplanted to dog, rabbit or rat; however, should a tumor develop in the new host following such a transplantation, there is the possibility that it may have been a spontaneous growth; it is very improbable that the growth was derived from the heteorogenous cells. In the case reported by C. Lewin many years ago, the transplantable tumor, which formed in the rat following transplantation of pieces of human cancer, was of a very low degree of specificity and was apparently constituted of cells which usually take a prominent part in inflammatory reactions.

In heterotransplantations of normal tissues we have seen that the toxic action of the bodyfluids of the host is much more evident in the destruction of the transplant than in homoiotransplantations. Although in the latter the toxicity of the bodyfluids does injure the transplant, the cellular and vascular reactions of the host are, here, relatively more important. Likewise, heterotransplanted tumors are primarily injured by the heterotoxins of the bodyfluids of the host, although cellular reactions may secondarily participate in the destruction of the graft.

Since, after homoiotransplantation of a tumor, even in an animal immunized against it, it is usually the central part of the graft which dies first, while after heterotransplantation in an immunized animal the peripheral, as well as the central, part shows signs of injury, it has been assumed that immune substances exert their injurious action only on heterotransplants, and

usual active way without undergoing regression. This conclusion is also confirmed by the fact that regression of a Walker rat carcinoma or a Jensen rat sarcoma did not cause immunity against the growth of the Ehrlich-Putnoky tumor. If the carcinomatous cells had assumed, at least partly, the species differential of the rat, the immunity against the growth of a rat tumor caused by the previous retrogression of a rat tumor in rats should have affected also the Putnoky tumor. On the other hand, the growth of the Ehrlich-Putnoky rat-strain tumor in rats can be prevented by previous inoculation of mouse embryo-skin into the rats. This again indicates that the tumor cells still possessed the species differential of the mouse. If inoculation of mouse embryo-skin induces only a slight retardation of the growth of the Ehrlich-Putnoky tumor in the mouse, this is presumably due to the very strong growth momentum which this tumor possesses in mice. In a similar way, Purdy found in heterotransplantation of fowl tumors into ducks, ducks were made immune to the fowl tumors by a previous implantation of chick embryo, but not of duck embryo.

However, there is one observation which might suggest that an actual change in the species differential of the Ehrlich-Putnoky tumor growing in rats has taken place. After regression of such a rat-propagated tumor in rats, the latter have acquired an active immunity against the growth of subsequently inoculated Walker and Jensen rat tumors; rat-propagated Putnoky tumors are more effective in this respect than are Ehrlich mouse-strain carcinomas. But this difference may perhaps be due to the fact that the Ehrlich-Putnoky rat-strain tumors grow much more vigorously in rats than do mouse-strain tumors; they may therefore be expected to induce a higher degree of immunity. While there is no reason for assuming that the genetic constitution of the mouse carcinoma cells has been transformed into the genetic constitution of the rat, or that the better growth of these cells in the rat was made possible through a gene mutation or chromosomal change in these somatic cells, still it is possible that certain adaptive changes have gradually taken place in the Ehrlich-Putnoky tumors, which make them more able to overcome the injurious conditions existing in the rat after long-continued propagation in this strange species. Also, in other series of heterotransplantations, adaptive changes of this kind seem to have occurred. These may consist in a gradually increasing growth energy of the tumors in the rat, or in an increasing resistance to the injurious rat substances, perhaps caused by a more active absorption and neutralization of such substances by the growing tumor cells. That such an adaptation may take place after heterotransplantation is indicated, also, by the fact that at first the temporary growth of the Ehrlich-Putnoky mouse tumor was accomplished only in very young rats, in which the reaction against strange differentials is less marked than in adult animals, and that only in the course of continued transplantations in the rat did the tumor begin to grow well, at least for some time, also in young adult rats. In addition to these adaptive changes, it is conceivable that in the course of continued re-transplantations into rats those metabolic cell activities which lead to the production of the organismal differential, and which in the last analysis

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heterogenous tumor, one must use heterogenous tissue of the same species as that in which the tumor originated. For instance, if one wishes to immunize a rat against a mouse carcinoma, the rat must be inoculated with normal mouse tissue, and such an immunization cannot be accomplished by inoculating rat or guinea pig tissues into the rat. Correspondingly, an immunity against a homoiogenous tumor can be attained only by the inoculation of tissues from the same species: a mouse can be actively immunized against a mouse tumor only by means of mouse tissues. Now we know that in the tissues used for heteroimmunization there are present in addition to the heterodifferentials, homoiodifferentials; but evidently the presence of the heterogenous differentials in some way prevents the homoiodifferentials from becoming effective under these conditions. We see, then, that the organismal differentials play a role in immunization against heterogenous as well as against homoiogenous tumors.

The direct action of heterotoxins, and especially of immune heterotoxins—but not of homoiotoxins—on tumor tissue, can also be demonstrated in tissue culture. While, as Lambert and Hanes have found, rat sarcoma grows in vitro as vigorously in plasma from immune rats as in the plasma from normal or tumor-bearing, non-immunized rats, the immune heterogenous serum from guinea pigs immunized against rat sarcoma has a toxic action on this tumor in tissue culture. It also exerts such an effect on rat-embryo skin. Other authors, such as Gussio, Mottram and Rous, likewise were unable to demonstrate the injurious action of homoiotoxins in tissue culture. Furthermore, Rous, and subsequently Kross, did not succeed in demonstrating the existence of homoiotoxins by means of parabiosis, in which substances produced in one animal are supposed to be transferred directly to the circulation and thus to the tissues of its partner. However, as shown in a previous chapter, factors which complicate parabiosis and tissue culture make it necessary to accept negative results obtained by means of these methods with certain reservations. In the case of chicken sarcoma growing in vitro, A. Fischer found that not only plasma from naturally resistant fowl was without any inhibiting effect, but even the serum of geese, ducks and rabbits immunized with chicken sarcoma did not prevent the proliferation of this tissue in vitro. However, the lack of effects of homoiotoxins in tissue cultures may, at least partly, be due to the fact that while in the living vertebrate organism the bodyfluids are constantly circulating and new substances of a specific character are carried to the transplanted tissue, in vitro the amount of such an injurious substance which is able to act on the tissue is very limited, and the tissue can presumably neutralize it to some extent; the results obtained in vitro are, therefore, not comparable in every respect to those obtained in the living animal.

In a preceding part it could be shown that the homoiotoxins in the blood stream of a homoiogenous animal may injure directly the transplanted normal cells. This is more noticeable in some species than in others, and especially it is noticeable if the difference between the individuality differentials of host and graft is relatively great and if the transplanted tissue is sensitive. There are indications that immune substances may be formed as a result of a first transplantation, which cause an acceleration of the reaction against the

not on homoiotransplants. However, the primary degeneration of the more central parts of a homoiotransplant is due to the more unfavorable condition of these areas, involving a deficiency of oxygen and possibly also of other foodstuffs during the period directly following transplantation; the central portions of the tumor may therefore be more accessible to the action of the injurious homoiotoxins, while in the peripheral parts these toxins are not strong enough to destroy tissues which live under relatively favorable conditions. On the other hand, the more active heterotoxins, especially the immune heterotoxins, may accomplish a direct injury also of the peripheral parts.

The reaction against homoiogenous tumor transplants depends, at least in part, on the development of an active acquired immunity in the host; whereas, in the case of normal homoiogenous tissue transplants, the injurious effect seems to be due largely to the action of primary homoiotoxins and to the direct response on the part of the host tissues. Similarly, while in the case of heterotransplanted normal tissues the preformed heterotoxins and the activity of the cells of the host play the principal role, and immune heterotoxins seem to enter into the reaction only secondarily, in the case of heterotransplanted tumors the effect of active immunization can be more readily demonstrated. Through a previous inoculation with normal tissues from the heterogenous species to which the tumor belongs, immunization of the host can be accomplished and the destruction of the tumor transplant can be much accelerated. While under these circumstances the action of the immune heterotoxins is the most important agency that causes the rapid destruction of the transplant, an intensified reaction on the part of the lymphocytes may play a part here, as well as after homoiotransplantation of tumors into actively immunized animals; it is by means of this accumulation of cells around the graft, rather than by a lack of ingrowth of connective tissue and blood vessels from the host into the transplant, that the incompatibility between the differentials of the host and transplant may become manifest.

We may then conclude that in the case of tumors, as well as of normal tissues, it is primarily the primary, performed heterodifferentials which call forth the reaction of he host tissue against the transplant, and that it is these heterotoxins which injure the transplanted tumor. Secondarily, such heterodifferentials may act as antigens and call forth the production of immune heterotoxins, which are especially effective in the case of tumor transplants; associated with this process may be an intensification of the cellular reaction. Tumors are the descendents of normal tissues; they have retained the organismal differentials of the latter in all essential respects ,and they call forth, therefore, the same primary reaction in the hosts. But some changes take place in the normal tissues during their transformation into tumors and it is in consequence of these changes that tumor tissues differ in certain respects from normal tissues in their transplantability and in the reactions they call forth in the host.

That, however, notwithstanding these modifications the organismal differentials of the tumors play a significant role in transplantation, is also made evident by the fact that in order to accomplish an immunization against a

of a sarcoma emulsion on which it had acted for a few hours. Similar extract, in which serum of normal rats had been used, was ineffective.

The experiments of Lumsden are in accordance with the conclusions at which we arrived in our experiments with normal tissues, namely, that primary (natural or preformed) heterotoxins in the host exert a direct injurious effect on the transplant; the presence of the heterodifferential in the transplanted tissues is responsible for the accumulation of lymphocytes and polymorphonuclear leucocytes and for the marked development of fibrous tissue around the graft. Various observations have made it probable that secondarily there is superimposed upon the natural preformed heterotoxin, a secondary immune heterotoxin, which is especially readily demonstrable in the case of tumor grafts. There is reason for assuming that the heterodifferentials may act as antigens, which lead to the production of the immune heterotoxins.

Experiments in heterotransplantation into related species have been carried out, not only with mammalian tumors, but also with certain of the filterable chicken sarcomata and related fowl tumors which can be transmitted to other birds by the inoculation of tumor cells as well as by means of an agent separable from cells. Fujinami transferred his chicken myxosarcoma to ducks and propagated it here serially through forty generations. Gye, by means of filtrates or of cell suspensions, could transmit the same tumor serially to ducklings, but in half-grown or adult ducks the tumor could grow only for some time and it later regressed. But Purdy succeeded in transmitting it serially to ducklings as well as to adult ducks by injecting very large amounts of minced tumor tissue. There is some reason for believing that the chicken tumor cells, as such, were able to grow in the heterogenous host, because it has been found possible to elicit a certain degree of immunity against the fowl tumor by a previous injection of minced fowl-embryo into the ducklings. It is of interest that a chick in which a Fujinami tumor happened to regress, had thereby acquired an immunity against a Fujinami sarcoma but not against a Rous tumor.

As to the heterogenous transfer of Rous chicken sarcoma, Purdy was not able to accomplish this in adult ducks by injection of large amounts of virus-containing extracts, but by transmitting large quantities of minced tumor tissue he could transfer Rous sarcoma through several generations of very young ducklings; he was unsuccessful in similar experiments with adult ducks. Des Ligneris likewise had negative results when he used adult ducks and also geese, but he succeeded in transferring the tumor to turkeys and guinea fowls. Not all such chicken tumors, however, can be transferred into foreign species; growth did not take place with Begg's endothelioma. If it is then probable that at least in some of these instances we have actually to deal with a successful transplantation of tumor cells into heterogenous hosts, we must not lose sight of the fact that host and donor belonged to relatively nearly related species, and furthermore, that it is not ordinary tissue cells which developed, but cells stimulated to grow by an agent which has invaded them; moreover,

transplanted tissues. More marked than the injurious effect of the homoiotoxins is that of heterogenous bodyfluids, containing heterotoxins, on transplanted tissues; these heterotoxins damage all transplanted tissues without the cooperation of host cells. The action of sera on tumor cells has been studied particularly by Lumsden and his coworkers. They distinguish: (1) homoiotoxins which develop, for instance, in a rat after immunization with Jensen rat sarcoma and rat spleen. They injure cancer and normal wandering cells from the same species in tissue culture, but not other kinds of normal tissue cells. These substances are heat-labile and are contained in the euglobulin fraction of the serum; (2) normal heterotoxins which are injurious for cells of all other species. They are very heat-labile and are also contained in the euglobulin fraction; they can be increased in quantity through immunization; (3) immune heterotoxins which are directed specifically against the species which has been used for immunization. These are stable, heat-resistant, and are contained in the pseudoglobulin fraction; (4) and possibly in addition to the species-specific antibodies, tissue- or organ-specific immune substances. Lumsden believes that there is evidence as well that "anti-malignant" immune substances develop in response to inoculation of cancer tissue into animals of the same species in which the cancer originated, for instances, in response to inoculation of the Jensen rat sarcoma into rats. These sera would act not only on the kind of cancer which was used for immunization, but also on various other types of cancer, irrespective of the species in which they originated. However, other investigators (Phelps) find that such sera are not specific for cancer cells, but contain heterotoxins which act equally well on normal cells of the species to which the antigen belonged. Lumsden, Macrea and Skipper themselves noted that such "anti-malignancy" sera kill also young, not as yet much differentiated macrophages emerging from spleen cultures; however, these sera do not affect the macrophages of the producer of the antiserum. This is presumably due to the fact that in the latter case we have to deal with autogenous cells, while the ordinary "anti-malignancy" sera act either on homoiogenous or heterogenous cells. At present it appears doubtful whether such anticancer sera exist. However, it is very probable that heterogenous, and also homoiogenous cancer growth in an animal may call forth the production of immune substances much more actively than do normal adult tissues; but strange embryonal tissues likewise produce immunity, and it is very probable that the more active growth of cancerous tissue as compared with inoculated adult normal tissue is at least one of the factors that is responsible for the difference in the effectiveness of these various tissues serving as antigens.

By a different method Woglom attempted to prove the existence of immune substances in the serum of rats inoculated with rat sarcoma 39, after the spontaneous retrogression of these tumors. He absorbed the immune substances which were present in the blood of these rats by means of a mash of sarcoma 39. After subsequent extraction of the immune substances from the sarcoma mash with Locke solution, this extract inhibited the growth

spleen and other organs were fully differentiated, the growth ceased and the transplant died. From more recent experiments, it appears that not only the allantoic membrane, but also the yolk-sac of the chick embryo, is suitable for the growth of the heterogenous tumors (Taylor, Thacker and Pennington). Shirai and Murphy noted a better growth of heterogenous tumors in the brain than in the subcutaneous tissue; the lymphocytic reaction was diminished in these instances if contact with the meninges was avoided. Greene and Saxton succeeded in transplanting into the anterior chamber of the eye of rabbits, homoiogenous tumors which failed to grow when the usual modes of transplantation were tried. In 1937, a Russian investigator, Smirnova, observed that human and mouse tumors grew from four to six months in the anterior chamber of the eye of rats. Greene carried out successful serial transplantations of rabbit tumors in the anterior chamber of the eye of guinea pigs; a human fibrosarcoma, and even a human scirrhous cancer of the mammary gland, grew in this organ. The chick chorio-allantoic membrane, the brain and the anterior chamber of the eye represent places where the aggressive reactions on the part of the host are diminished. In a previous chapter we have discussed already, to some extent, the factors which make possible a better growth of heterogenous embryonal and tumor tissues in these places and we shall return to this problem later, when we analyze the processes of immunity which develop againts transplanted tumors. In this connection we may refer also to long-continued growth of benign and malignant tumors in roller-tube tissue cultures, in which the medium consisted of coagulated chicken plasma covered by human serum (Gey, Coman).

Also by other means it was possible to improve the growth of tumors in hosts bearing strange individuality or species differentials. Thus it was found that after previous irradiation of the host by a sufficient dose of X-rays the resistance of the latter against the growth of homoiogenous as well as of heterogenous tumors was diminished (Murphy, Clemmensen and others). Moreover, the transfer of mouse leucosis to otherwise unfavorable hosts could be promoted by these means (Krebs, Furth). Not only tumor and leukemic cells could thus be transferred more successfully, but also Shope's rabbit fibroma virus, when injected into X-rayed or tarred rabbits, caused generalized fibromatosis, the tumors showed a prolonged growth in these animals and in one case the fibroma assumed a sarcomatous character (Andrewes). According to Maisin and Masse, also minced embryonal chick tissue develops, in chickens previously treated with tar, into larger embryomata, which persist for a longer time. Injections of trypan blue and of other colloidal substances (Lignac, Ludford, Andervont) diminish the resistance to the growth of tumors in homoiogenous hosts, and in addition, trypan blue inhibits the development of some types of immunity (Andervont).

These various agents, X-rays, colloidal dyes and tarring, act presumably on the reticulo-endothelial system, the usual place for the production of generalized immune reactions. On the other hand, the possibility must be considered that also the primary reactions against strange normal tissue, tumor or virus, due to the presence of preformed substances, may depend upon the reticulo-

it is of importance to note in this connection that cells of a less differentiated nature composed these tumors. Moreover, different types of fowl tumors differed in their ability to grow in heterogenous hosts and, likewise, different races of certain avian species differed in their suitability as hosts. The transfer of the tumor by large amounts of tumor cells was, on the whole, more successful than the transfer by means of virus-containing tumor extracts. In general, fowl sarcoma could be transmitted much more readily to newly hatched heterogenous birds than to somewhat older ones.

According to the quite recent experiments of Duran-Reynals it can be shown that if we transfer Rous fowl sarcoma I to ducklings, at first the unchanged chicken tumor virus or cells cause the tumor development in these ducklings, as indicated by the fact that the tumors thus produced have not only the same morphological characteristics as the original chicken tumor, but they also possess the same tissue affinities and the same tendency to develop in certain regions of the host. However, after the tumors have lived for some time in ducklings, they may change their characteristics, the chicken-adapted virus becoming duck-adapted virus; it tends to cause sarcomas in organs, different from those in which it grew at first, growing now in bones or lymph glands, calling forth a lymphosarcoma in the latter organ. Furthermore, this changed tumor tends to become generalized. A similar adaptation seems to occur if tumor cells are inoculated; these also cause the same kind of tumors as the duck-adapted virus. Such a duck-adapted virus or cell suspension induced not only tumor formation in ducklings, but also in adult ducks. Cells and viruses are now no longer heterogenous but homoiogenous elements for the duck. If such duck-adapted virus or cells suspension is transferred back into chicken, it seems at first to behave like material heterogenous for the chicken, but after some time, the duck-adapted virus or cell suspension can again become chicken-adapted, being thus converted into a homoiogenous virus for the chicken. However, it should not be concluded from these experiments that a chicken cell was actually transformed into a duck cell, but it seems, merely, that the changed virus altered secondarily the tumor-producing characteristics of the chicken cells in which the virus lived.

We have seen that it is possible to a certain extent to protect transplanted normal tissues against the injurious reactions which ordinarily take place in the host, and by various means to diminish the reactions in the host which follow as a rule transplantation of strange tissues. Thus homoiotransplants induced less active reactions when younger hosts were used; also when they were made into the anterior chamber of the eye, into the brain, or after preceding injections of trypan blue. More striking differences have been observed when similar methods were employed in the case of transplanted tumors, and, indeed, experiments with tumor transplants preceded experiments of a like nature with normal tissues. It has been found by Murphy that transplanted heterogenous tumors, even if host and tumor were phylogenetically far distant, grew for some time on the chorio-allantoic membrane of the chick; an experiment with heterogenous embryonal tissue also succeeded; but as soon as the development of the chick embryo had reached the stage at which the

tures, but a part of the tumor itself. The tumors grew, as a rule, only in the same animal in which they originated and not in other animals of the same species. The individuality differential, therefore, asserted itself under these conditions. This was observed in some of our experiments, as well as in those of Ribbert and Mann, but we, as well as subsequent investigators, found that different benign tumors may differ in their power of resistance to homoiotoxins, it being possible to transplant certain of them serially into other individuals of the same species. We observed, furthermore, that while after transplantation of carcinoma or sarcoma, the greater part of the transplant became necrotic and only a small peripheral zone remained alive, in the case of these benign tumors of the mammary gland a greater portion of the peripheral tissue could be preserved, and in some autotransplanted tumors even almost the whole of the transplant; evidently some of the constituent parts of the tumors, especially the fibrous ones, were more resistant than very cellular and rapidly dividing malignant cells. In addition, while with malignant tumors as a rule, an increase in growth energy occurred in the course of the first few transplantations, such an increase was lacking with these adenofibromata. In our experiments there was a gradual decline in the growth energy after successive transplantations. Another difference between these two types of tumors consisted in the different effects which hormones exerted on their growth. Cancerous tissues, in particular carcinomas of the mammary gland, are no longer accessible to the action of ovarian hormones, whereas a positive effect was quite evident in the case of benign tumors of the mammary gland; such tumors retained the ability to respond with marked growth processes to the action of hormones, which determine the growth processes in the normal breast tissue during pregnancy. The cells of these adenofibromata evidently had not changed their physiological characteristics to the same extent as the cells of malignant mammary gland tumors. Moreover, while in our experiments the tumors were propagated mainly in female rats they were able, also to grow in male rats.

It may therefore be concluded that the fate of the transplanted benign tumor depends not only on the organismal differentials, but also on its mode of growth and some other factors, which are localized either in the tumor cells themselves or are circulating in the bodyfluids of the host. Some of the factors localized in the tumor cells correspond to those present also in normal tissues, in particular, the organ or tissue differentials, which help to determine whether a tissue is able to withstand the injury connected with autotransplantation.

A further factor in determining transplantability is the increase in growth energy acquired by normal tissues during their transformation into benign tumors. This additionl growth is relatively slight, although it varies in different benign tumors. Correspondingly, the morphological and biochemical modifications, which the normal tissues undergo during their change into benign tumors, are less marked than those which take place during their change into malignant tumors. In this respect again, different benign tumors may behave somewhat differently, and it should therefore be expected that they show a

endothelial system. This is perhaps indicated by the fact that the lymphocytes and leucocytes of the blood may react as early as within the first few days against a homoio- or heterotransplant of normal tissue or tumor.

But there are several observations which indicate that the application of X-rays, injections of tar, or perhaps even of trypan blue, may have results of a quite different kind; they may promote the induction of autogenous cancers elsewhere in mice under the influence of tar or other carcinogenic substances (Mayneord and Parsons, Maisin and Masse, Andervont). A related phenomenon is probably the effect of X-rays or tar on the development of rabbit fibroma following the injection of virus mentioned above. In addition, Rous and his collaborators produced carcinomata in rabbits through intravenous injection of rabbit papilloma virus, in places where the skin had been irritated through previous applications of tar. These effects cannot be due to an inhibition of immune processes developing against strange tissues or tumors and their organismal differentials, but mechanisms of a different kind must be active in these experiment.

Taken altogether, these experiments add further data in support of the conclusion that in principle the host reacts in a similar way against normal tissues and against tumors, but that secondary factors may be added in the case of tumors, which may induce certain modifications in the types of reaction which occur; and furthermore, that it is the organismal differentials which normal tissues and tumors have in common.

Transplantation of Benign Tumors

We have, so far, analyzed some of the principal factors which determine the growth of transplanted malignant tumors, with particular regard to the significance of organismal differentials of host and transplant. It will be of interest, now, to compare with the growth of cancerous tissue, that of benign tumors. In experiments beginning in 1901, and continuing at various periods during the course of the following thirteen years, we transplanted, at various times, altogether four mammary fibroadenomata and two mammary fibromata of the rat, and, with S. Leopold, a mixed mammary tumor, a chondromyxadenoma of a dog. Similar experiments were subsequently reported by Ribbert, Borrel and Petit, and more recently, by Mann, Robinson and Grauer, Heiman, Heiman and Krehbiehl, Umehara, Picco, Oberling, and the Guerins, Emge and Wulff, as well as by Wolfe, Burack and Wright.

From our investigations the following conclusions may be drawn: Benign tumors show a reaction in certain respects intermediate between that of normal tissues, which after serial transplantation manifest at most only a very limited and transitory regenerative growth, and that of malignant tumors. If they grow at all, they usually do so only very slowly and after a relatively long preceding latent period, during which, however, mitotic proliferation may occur. In the majority of cases we had to deal with tumors of the mammary gland, which were composed of adenomatous as well as of fibrous constituents, and both could take part in the subsequent proliferation; the fibrous portion evidently did not represent merely the stroma of the epithelial struc-

of these fibroadenomas of the mammary gland under the influence of certain hormones may enable them to overcome the resistance to their growth, caused by unfavorable individuality differentials of hosts, and to grow, therefore, after homoiotransplantation. There were also some indications that there exist strain differences, a certain tumor growing better in one strain of rats than in another; presumably strains in which the individuality differentials of the rats were similar to that of the tumor were more suitable than strains with more strange individuality differentials.

While as the result of the growth or of the retrogression of transplanted malignant tumors the bearers of the transplant may become immune against a second transplantation, such an immunity has not been observed in the case of benign tumors; the latter behaved in this respect, as well as in the lack of adaptive processes and in their responsiveness to hormones, similar to normal tissues, while as far as the growth energy and abnormal mode of growth is concerned, they are intermediate between normal tissues and cancers. In cancerous tissue the inner growth factor (Gi) has become so strong and provides so stable a growth momentum to the tumor cells that extrinsic factors (Ge), such as hormones, have no longer any chance to affect the growth to any marked extent. In normal tissues and benign tumors the relation between Gi and Ge differs in favor of Ge, whereas in cancerous growth Gi predominates; in addition, other changes may have taken place in the tissues during their cancerous transformation, which tend to diminish the effectiveness of regulatory processes. It is then the strength of Gi (endogenous growth factor) which is one of the factors enabling a cancerous tumor to overcome difficulties in transplantation, and especially in serial transplantation, and perhaps the main difficulties in the way of the transplanted cells consist in differences in the individuality and species differentials between host and transplant. In tumors in which Gi has not yet reached sufficient strength, there is therefore need for the additional and longer continued action of Ge (exogenous growth factor) and this applies especially to certain benign tumors, where hormones may affect favorably the growth of transplanted fibroadenomas. Also, in certain other tumors which have not yet reached a fully cancerous state, the effect of Ge, acting on the host of the graft, may be required for a successful growth of the tumor in the strange host; thus Gardner noted that estrogen administration in male mice could make possible the development of transplanted tumors of the interstitial gland of the testicle. Although such tumors could occasionally metastasize, on the whole they had reached only a very low degree of growth momentum, or, expressed differently, they had not yet progressed very far in the process of cancerization. They therefore could be transplanted successfully only if the host received, at the same time, estrogen. The same factor, Ge, which helped to cause in the normal tissue the increase in Gi and thus the transformation into cancer, was needed in order to add to the growth momentum of the transplant and aid it in surviving and growing under otherwise not quite adequate conditions. There seemed to exist a somewhat similar action of Ge in the transmission of leukemia from hybrids between mice belonging to a leukemic

different degree of transplantability. This is indeed a fact, as our own experiments have already indicated and as the subsequent, more extensive experiments of various investigators, especially those of Heiman, Emge and Wolfe, and their collaborators, have shown. Thus certain benign tumors cannot even be autotransplanted, while some others can be homoiotransplanted through a number of generations. But when homoiotransplantation does succeed, the latent period is usually long and the subsequent growth very slow.

Fibrous tissue was a relatively prominent constituent in many of the benign tumors which so far have been used by various investigators for homoiotransplantation; it surrounds and protects the epithelial structures. It is very probable that homoiotoxins are not given off to any considerable extent by tissues of this kind, and hence accumulations of lymphocytes are not prominent after homoiotransplantation of such tumors.

Subsequent experiments, especially those of Heiman, of Emge, and of Wright and Wolfe, have contributed further data as to the influence of hormones on the growth of these tumors. Castration of the host had a marked effect, in the investigations of Heiman, and Heiman and Krehbiehl, castration of female rats lowering, and castration of male rats improving the transplantability. Furthermore, gonadotropic hormones, and still more so, combinations of these hormones with estrogen, and also estrogen alone, could promote very noticeably proliferative processes in these tumors, especially in castrated female and male, as well as in normal male rats. However, while application of these hormones was thus effective in intensifying growth activity in these tumors, and in some instances especially in their adenomatous constituents, it has not been possible so far to increase thereby the growth energy to such an extent that a definite transformation into a carcinoma took place. However, it seems that Wright and Wolfe succeeded, by means of estrogen injections for as long as 50 days or more, in producing proliferations in the epithelial parts of a fibroadenoma in rats, which seemed to be precancerous or perhaps represented beginning cancerous changes. On the other hand, both Heiman and Emge were able, in some tumors, to stimulate the growth of the connective-tissue constituent so markedly that a fibroma became converted into a spindle-cell sarcoma; it seems that this stimulation was the result of continued serial transplantations, but there are some indications that in some cases also stimulation by pituitary-like hormones exerted similar effects. The beneficial effect of gonadotropic hormones on the growth of the tumors also in castrated females suggests a direct action on the tumor rather than an action mediated by the sex glands. Androgenic hormones, on the other hand, tended on the whole to diminish the number of successful transplantations of fibroadenomata of the mammary gland; an action which is in agreement with the fact that castration in male rats, which means removel of the sex hormones, raises the number of takes of these tumors. According to Heiman, also progesterone inhibited the growth of the epithelial portion of the fibroadenoma, and it reduced the number of takes; still more effective in this respect was a combination of testosterone and progesterone.

It may be assumed that the intensification of the growth energy of some

Chapter 2

Heredity and Transplantation of Tumors

In the preceding chapter it has been shown that the results of autogenous, homoiogenous and heterogenous transplantations of tumors differ greatly and that the differences are very similar to those found in corresponding transplantations of normal tissues. In both tumors and normal tissues the organismal differentials are identical, or almost identical, in host and graft in case of autotransplantation; they are different in case of homoiotransplantation, and still more unlike in case of heterotransplantation. While these investigations have established, in a definite way, the importance of organismal differentials, and therefore also of heredity in the transplantation of tumors, there were already some earlier observations which pointed to the significance of constitutional hereditary factors. Thus Morau, in his transplantations of mouse carcinoma, believed that in the offspring of mice in which the tumors could be transplanted successfully, the chances for the growth of the transplanted tumor were better than in not directly related mice. At an early stage in our first series of transplantations of rat sarcoma, we found that this tumor did not grow in a strange species, even in one nearly related to the rat; neither did it grow in some white rats; but it did grow in a hybrid between a gray and a white rat. At that time we decided, therefore, to study the finer differences within white rats which determine their suitability or lack of suitability as hosts for these tumors. The presence of a constitutional element in tumor transplantation was also indicted by our observations that in the same individual in the case of multiple simultaneous transplantations of the same kind of tumor, either all or none of them took; and that if a transplanted tissue did not take in an individual rat, subsequent transplantations proved usually likewise negative, although some exceptions to this rule occurred, indicating that certain accidental, variable factors complicated these experiments. Similar observations were made by Jensen in his serial transplantations of mouse carcinoma. Subsequently, Michaelis, as well as Bashford and Murray and others, found that white mice obtained from different localities differed in the number of takes which followed inoculation with mouse carcinoma. In the meantime, we had successfully transplanted a carcinoma, originating in a Japanese waltzing mouse, in about 100% of all Japanese waltzers, although the growth in the first generation of transplants was slow. This indicated that here we had to deal with a very favorable soil of a homozygous character. A few years later, Tyzzer (1909) studied the differences in the number of takes of another carcinoma, which had developed spontaneously in a Japanese waltzing mouse, after transplantation into Japanese, into common white mice, and into hybrids between these two species or subspecies. Tyzzer expressed the view that hereditary factors determine the differences in

and to a non-leukemic strain to the parent strains; this transmission could be accomplished only in the parent belonging to the leukemic strain, perhaps because here a factor favorable to the development of leukemia was active.

All the data which have been considered so far lead, then, to the conclusion that in normal tissues and benign tumors, as well as in cancers, the organismal differentials are present and affect the results of transplantation, but that in cancers the increase in inner growth momentum and perhaps other changes which make possible an increased adaptation to environmental conditions in the host, may help to overcome the unfavorable effects of strange organismal differentials.

The essential similarity between the organismal differentials of tumors and normal tissues, and the similar significance which both of these differentials have in determining the reactions of the host against transplants have been made evident also by the recent investigations of H. T. Blumenthal, who studied the effects of various types of transplants on the character of the white blood cells in the circulating blood. We shall discuss his findings more fully in the fourth chapter of this part, where we analyze the cellular reactions in the bodyfluids which develop against tumors.

The transition from the ordinary strains of animals to closely inbred, homozygous strains is a gradual one; it takes place step by step. The guinea pigs and rats which we used at first in our transplantations of normal tissues did not belong to inbred strains. We determined the organismal differentials by comparing the effects of auto-, various kinds of syngenesio-, homoio- and heterotransplantations. Because we did not have to deal with closely inbred strains, variability in the results within certain limits in the syngenesious-homoiogenous range of the spectrum of relationships may therefore be expected, and this was actually observed. But this difficulty could be overcome by increasing the number of experiments in which we tested the effects of relationship on the fate of the transplants. The greater the number of unknown factors, the greater must be the number of equations. The conclusions reached in these earlier experiments concerning the significance of the relations between organismal differentials of host and transplant on the fate of the latter were confirmed in our subsequent investigations with closely inbred strains of guinea pigs and rats. However, a fully homozygous condition had not yet been reached in the case of the inbred guinea pigs; in the case of the rats, the heterozygous condition had only very slightly been diminished after as many as forty generations of close sister-brother inbreeding. As stated, our early observations on the transplantation and spontaneous development of tumors in mice were made in partly inbred strains. The same limitations applied here as in the earlier transplantations of normal tissues in guinea pigs and rats. In both instances, the difficulties due to the larger number of variable factors present made necessary a larger number of experiments. Likewise, in the case of tumor transplantations subsequent experiments by various investigators and also by ourselves with more fully homozygous strains confirmed essentially the earlier conclusions. It must, however, be emphasized that even these closely inbred strains had, in all probability, not yet reached a completely homozygous condition. There is therefore only a quantitative difference in the nature of the strains used in the earlier and in the later investigations, and both lead to the same results provided a sufficient number of experiments are made.

All these observations and experiments point to the conclusion that the transplantability of tumors depends largely on the relations between the genetic constitutions of host and donor and the character of the organismal differentials, which is the expression of these constitutions. But there is one finding which seems contradictory to these conclusions. Rous and Long discovered that their third chicken sarcoma, which had originated in a Leghorn, grew, on the average, better after transplantation into Plymouth Rock chickens than in Leghorns. Presumably factors of a secondary character complicated the relationship between tumor and host in this case, or this condition may possibly have been due to peculiarities of the agent present in these tumors rather than to those of the tumor cells. But before entering into a further discussion of genetic factors in the transplanted tumors, we must again consider the difficulty which we experience if we analyze tumor growth by means of transplantation of the ordinary transplantable tumors.

suitability of the hosts for the tumor he used; however, in his experiments he had to deal with mice which belonged to different subspècies. Cuénot and Mercier separated, through breeding of white mice from their locality, families in each of which the degree of transplantability was a fixed quantity which was inheritable; they believed that, through breeding, they had been able to sort out what corresponded to Johannsen's pure lines. But such investigators as O. Hertwig and Poll, and C. Lewin, denied the existence of these differences of susceptibility to tumor transplantation between different families or strains within the same species, and it was especially an experiment of Haaland which was responsible for the assumption, subsequently made, that adaptive variations in the animals, taking place in response to changes in the environment, rather than fixed constitutional characteristics were the cause of these differences between different strains. Haaland observed that mice bred in or near Frankfurt, which were suitable as hosts for Ehrlich's mouse sarcoma, became unsuitable after they had been transferred to Norway and bred there for a short time. He attributed this change to the difference in the kind of food given to the mice in these two localities and concluded, therefore, that the suitability of hosts for a certain tumor depended on variable environmental, rather than on fixed inheritable conditions. Other investigators confirmed Haaland's observations and accepted his conclusions as to the effect of various kinds of food on the number of takes of a certain tumor.

On the other hand, our investigations, made in conjunction with M. S. Fleisher (1912), showed that the differences in transplantability occurring in different strains of mice depend upon fixed hereditary conditions, which are independent of environmental factors. American and different types of European white mice, all fed in the same way and bred separately under identical environmental conditions, each maintained its characteristic transplantability index for a carcinoma which had developed spontaneously in an American mouse. Subsequently Morpúrgo, and also Roffo, made similar observations. The change which Haaland found in his mice after transfer to Norway was interpreted by us in a different manner, because we noted that in one of our European strains a change in its suitability as host took place as a result of a disease which eliminated a number of families. Evidently a selection had occurred, causing the survival of a family which differed genetically from the rest, and which now began to predominate over the other mice. As a result of this selection process, the transplantability rose considerably in this strain. However, the results of transplantation depend not only on the host, but also on the kind of tumors which are used for inoculation. Thus Haaland noted that if each mouse is inoculated with two different types of tumor, the receptivity of different strains of mice differed for each tumor. As we may now express it, the transplantability depends upon the relation of the individuality or organismal differentials of the host to those of the transplant. But we must make the reservation that, within a certain range, adaptive changes may take place *in the tumor cells* and that thereby the results may be modified.

mutual suitability of hosts and grafts than do differences in the number of takes. This was quite evident, for instance, in some of our experiments in which mouse carcinoma IX, in the beginning, grew in all the mice inoculated and the number of takes was, therefore, 100 per cent; for a time following transplantation, also the growth energy was approximately the same, or at least similar; but after a certain size had been reached by the transplants the growth energy diminished in some of the animals, the tumors retrogressed and finally disappeared, while in others they continued to grow. The main distinguishing feature between the tumors in the different hosts was the development or accumulation in some of the animals of certain unfavorable factors, which caused a slowing of the growth or even a retrogression of the tumors, while in others, conditions were more favorable and the growth energy was not markedly diminished.

To return now to the study of the conditions which determine the results in transplantation of tumors, Tyzzer (1909) hybridized two strains of animals, one of which was very favorable and the other very unfavorable to the transplantation of a certain tumor. In a Japanese waltzing mouse a tumor developed, which grew in 100 per cent of Japanese mice but not at all in white mice. As stated above, the Japanese waltzing mice have apparently become a relatively homozygous strain or subspecies. Tyzzer found that in the F_1 hybrids between the Japanese and white mice the tumor grew as well or even better than in the Japanese mice, whereas in the F_2 and F_3 hybrid generations no growth took place. As we have already stated, it is not possible to analyze the individuality differentials if we use one of the readily transplantable tumors, because these tumors grow in many animals of the same species, without regard to differences in the individuality differentials; there may, however, be some differences in the average number of takes in different kinds of strains, in which the averages of individuality differentials are different. However, Tyzzer in his series of transplantations did not actually study strain differentials, but something akin to subspecies differentials. He concluded from his experiments that the inheritance of factors which determined the transplantability of tumors did not take place in accordance with Mendelian principles.

In the following year (1910) Cuénot and Mercier, to whose investigations we have already referred, were concerned with the inheritance of the factors influencing tumor transplantability in white mice. They believed that it was possible to sort out, in these animals, pure lines in which the average transplantability of a certain tumor was a fixed quantity; furthermore, they believed that the extent of the deviation from this mean was likewise a characteristic feature for a pure line. The pure line to which a mouse belongs determines the percentage of cases in which a tumor can be transplanted; on the other hand, the character of the parents does not necessarily indicate whether a transplanted tumor piece will grow in a child; this may depend on phenotypic rather than on genetic conditions. However, in the light of what we now know, it is more difficult to obtain pure, fully homozygous strains even through long-continued close inbreeding, than should be assumed on theoretical grounds, and it is therefore improbable that Cuénot and Mercier

In the case of normal tissues we used as criteria of the suitability of the transplant for the host, or of that of the host for the transplant, the changes which the graft underwent in the host and the reactions of the host tissues, especially the lymphocytes, connective tissue and blood vessels, against the transplant. In this way we could show that there is a close correspondence between transplantability and the individuality differentials of host and transplant, as determined by the genetic constitution of host and donor. In the case of tumors, conditions are somewhat different. In general, in these experiments tumors are used which are easily transplantable, which means that they grow readily in a large number of individuals of the same species. Finer individual differences in relationship, such as those between parents and children, and between brothers, and even between somewhat farther distant relatives such as those tested by us in the case of normal tissues, cannot be distinguished if such transplantable tumors are used. The behavior of the latter towards different individuals belonging to species and strains in which they are readily transplantable, is to all appearances about the same. For this reason, only very marked differences between different hosts can be discovered in this way; they represent either strain differences or even differences as great as those between subspecies, or those obtaining between hybrids which result from the mating of two different strains. Furthermore, the standards used for the determination of the degree of transplantability are different for tumors and for normal tissues. For the latter, a more delicate grading of the suitability of the individuality differentials of host and transplant is made possible by the evaluation of the histologic changes which take place in the cellular reactions of the host against the graft. These criteria are not commonly used or available in the case of tumor transplantations; instead, the number of growing tumors (takes) and, less frequently, the growth energy of the tumors are employed as criteria of the differences in individuality differentials. In the investigations of M. S. Fleisher and the writer, a comparison was made between the averages of growth energy and number of takes as criteria of transplantability; in addition, we noted the duration of the latent period, that is, the length of time which intervenes between the date of transplantation and the appearance of the tumor visible to the naked eye. The length of the latent period measures the growth energy in the first and most critical period following grafting. It was found that the conditions determining the number of takes are in certain respects distinct from those determining growth energy, while in other respects they are correlated with each other. Both these factors depend primarily upon the relations of the organismal differentials of host and transplant, and secondarily, upon the growth energy of the graft and upon the adaptability of the transplant to different hosts or of the host to different transplants. However, tumors which may all be classed as transplantable, may still differ as to their growth energy; thus, in two parallel series of experiments, all inoculations may be followed by growth of the graft and therefore be considered as takes; yet, in one set of hosts the growth energy may be greater than in the other. In general, differences in growth energy in a certain host represent finer gradations of the

between American white and gray wild mice the differences between successive generations noted in our first series did not occur.

Subsequently Tyzzer and Little, and Little and Tyzer, found that a Japanese mouse sarcoma, as well as a carcinoma, grew in F_1 hybrids between Japanese and white mice as well as in pure Japanese mice, whereas in white mice the tumors grew in only a small minority of the animals. The growth of carcinoma and sarcoma behaved in these respects almost alike, but the growth of sarcoma was somewhat better. Also, Tyzzer and Little interpreted now, these variations in the percentage of takes in different generations of hybrids as due to the action of multiple factors. They assumed that the continued growth of both the sarcoma and carcinoma depended upon the presence of a complex of independently inherited factors, and this factor-complex was supposed to be present in a nearly homozygous condition in the Japanese waltzing mice. Since F_1 hybrids had Japanese mice as one of their parents, they possessed the factors comprising the Japanese complex in a single dose, and since this single dose allowed tumors to grow, it followed that a single representation of these factors was all that was required for the establishment of susceptibility to tumor implantation. Also, in his more recent investigations Little assumed that the transplant must have double representation and the host single representation of the genes required for continuous growth of the grafted tumor. It was furthermore necessary to hold that inasmuch as there was associated with the single complex of genes inducing susceptibility a set of unlike genes in the host, the set of genes determining susceptibility was dominant over the other set. Susceptibility was therefore supposed to be dominant over non-susceptibility or resistance to the growth of transplanted tumors. In addition, Tyzzer and Little assumed that the percentage of individuals in which the tumor grew in the F_2 generation could be used as an index of the number of factors necessary for continued growth. The larger the number of F_2 hybrids in which the tumor takes, the fewer the number of genetic factors required. For instance, these authors concluded that twelve factors were necessary in the case of a carcinoma, and from five to seven in the case of a more readily growing sarcoma.

In subsequent investigations into the transplantability of tumors, Little and Strong made use of strains which had been rendered more or less homogeneous (homozygous) by means of long-continued sister-brother inbreeding. In transplanting a melanotic tumor, which originated in strain dba, into hybrids between dba and A, Spangler, Murray and Little noted that transplantations succeeded in a larger percentage in colored than in albino hosts, and they assumed that one "susceptibility factor" is required for a successful transplantation in colored mice, while in albino mice there is needed, in addition, a second factor, which would be necessary also for melanin production in noncolored individuals but would function in this way only in the presence of the color factor. The use of closely inbred strains meant, in certain respects, a great simplification of the analysis of the growth of transplanted tumors and led to the establishment of some important facts in a more definite manner than had been previously possible. To mention only one example: Bittner, by

worked with pure lines. Levin and Sittenfield soon afterwards noted that the offspring of non-susceptible rats were less susceptible to the growth of a sarcoma than the offspring of susceptible rats.

In experiments with mouse carcinoma IX it was shown by Fleisher and the writer (1912) that this tumor could be successfully transplanted into a strain of American mice in 80 per cent of the cases, into a first strain of European mice in 23 per cent, and into a second strain of European mice in 3 per cent of the animals. However, in the early period following transplantation the tumors grew as well in European as in American mice, namely, in 85 to 95 per cent in both; but after twelve days a large proportion of the tumors became stationary and retrogressed in the European mice, while in the American mice the proportion of retrogressing tumors was small. In the F_1 hybrids between American and European mice the tumor grew as well or almost as well as in the American mice; but in the F_2 and F_3 generations there was a sharp fall in takes, which was followed again by a rise in the F_4 and F_5 hybrids. Except for the partial recovery in F_4 and F_5 and the results in back-crossing, our results and Tyzzer's were therefore similar. We concluded that these findings were compatible with Mendelian principles if we assumed that the susceptibility for growth in Tyzzer's, as well as in our experiments, depended on multiple factors: In this connection we applied the term multiple factors in the usual sense of the Mendelian theory, and in the same way in which we applied this term in our transplantations of normal tissues and in our analysis of the origin of tumors. In this sense we also explained subsequently the difference in the results obtained between transplantations of normal tissues from children to parents and in reciprocal transplantations from parents to children. In the former case, the number of genes which are present in the transplant but not in the host, should, on the average, be greater, and, on the average, the reaction should accordingly be more severe than in the reciprocal transplantations.*

In continuation of our experiments (1916), we extended our study to a number of other strains of mice. Again we found that American and various imported strains did not differ in respect to the number of original takes, but that they differed greatly in regard to the number of subsequent retrogressions. No marked individual differences in the growth energy could be established by the standards used at that time, either in that group of mice in which the tumor grew definitely, or in the other group in which it retrogressed. However, the marked differences which we had observed formerly in the number of takes or growth energy between different hybrid generations were no longer found; the growth throughout was about intermediate between that observed in the American and in the imported strains. Likewise, in hybrids

* In a paper by the writer on "The individuality differential and its mode of inheritance," in the *American Naturalist*, Vol. IV, Jan.-Feb. 1920, there occurs in the last paragraph of page 58, the sentence: "In the case of transplantation from child to mother, on the other hand, the graft would lack one-half the chromosomes—." It is quite evident that this is a misprint and that instead of "graft" it should read "host." This correction is made here, because this misprint has led to an erroneous interpretation of the views of the writer.

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means of this method, could show that if multiple inoculations of pieces from the same tumor were made into different individuals belonging to such a closely inbred strain, almost all of the inoculated pieces behaved alike in the same individual. Thus there was proved definitely the view the writer had expressed previously (1902), that all transplants from the same donor into the same host should elicit about the same reaction in the latter, the reactions depending, as we expressed it subsequently, on the relations of the individually differentials of host and transplants. Bittner furthermore found that if a closely inbred strain is used, the growth rhythms, described by Bashford as inherent in the character of tumor cells, do not occur. This agreed with the findings of Fleisher, who also had arrived at the conclusion that such rhythms do not exist.

Little, L. C. Strong, Bittner and Cloudman, noted that if a tumor originates in one of the inbred parent strains, it can be transplanted into all the individuals of this strain, but not, as a rule, into the individuals of another inbred strain. These observations are in agreement with the theory of the organismal differentials and accord with the earlier data established in experiments in which less closely inbred strains had been used. If two inbred strains are hybridized, a tumor which had developed in an animal belonging to one of the two parent strains grows well in all or almost all of the F_1 hybrids, while in the F_2 hybrids only a certain percentage of individuals is susceptible to the growth of the transplants in accordance with the rules of Mendelian segregation, and as mentioned above, this percentage figure, according to these investigators, can be used as an indicator of the number of factors which must be present in the hosts if the tumor shall take. The percentage of successful transplantations of the tumor into backcrosses between F_1 hybrids and each of the two parent strains indicates how many of the required growth factors in the hybrids have been contributed by each one of the two parent strains. As should be expected, according to the theory of the organismal differentials, a tumor which originates in an F_1 hybrid takes readily in all the F_1 hybrids, but not at all or very poorly in the parent strains, and it grows in a certain percentage of mice of the F_2 generation; this observation is also in agreement with the finding of Tyzzer that a tumor originating in a hybrid F_1 between Japanese and white mice, could not be transplanted into either of the parent strains. Strong compared the growth of two adenocarcinomata developing in two individuals belonging to the same inbred strain of mice. Because of the close inbreeding of this strain, we should have expected the tissues of the two adenomata to possess approximately the same individuality differentials; but Strong found that these two tumors behaved in a different way after inoculation into F_2 generations of hybrids between a strain of mice which was susceptible to the tumors and another strain which was non-susceptible. Therefore he concluded that the two tumors, although they had developed in individuals which should be expected to be genetically identical, differed from each other in their genetic constitution, and further, that two tumors structurally indistinguishable from each other may differ in their physiological behavior, an observation which in certain respects agrees with our own that

several spontaneous sarcomata which developed in the thyroids of different rats, differed very much in their behavior after inoculation into other rats, although these tumors were very much alike in their structure.

Continuing these experiments, Strong, as well as Bittner, studied two tumors which developed spontaneously in the same mouse of an inbred strain. These two tumors likewise were found to behave differently after transplantation into the same and into other inbred strains and into different generations of hybrids, and it was therefore believed that they differed in the number of genetic factors required for their continued growth in a strange host. Also, Cloudman, who transplanted three tumors originating in a mouse of the inbred A strain, and Bittner, who compared the growth of multiple tumors which developed spontaneously in an F_1 hybrid between the A and D strains, obtained similar results when the individual tumors were transplanted into A and D strains and into the different hybrid generations between A and D. But, although one of Strong's tumors grew in a larger number of individuals belonging to another strain and in hybrids between its own and the strange strains, otherwise the two tumors behaved in a parallel way as far as the relative percentages of their takes in these different kinds of mice were concerned. Both tumors were also affected in the same way by sex differences of the hosts after transplantation into F_1 hybrids, the females being the more favorable hosts.

Previously, Little had assumed that in female mice at the time of sexual maturity a change in the receptiveness to transplants occurs. He attributed the difference which he observed in the percentage of takes in newborn female mice and in mice three weeks old, to the sexual maturity which takes place during this period and to corresponding changes in the individuality differentials; this would represent a linkage between susceptibility and sex factors. But only in certain hybrid strains did the number of takes increase at the time of sexual maturity, while in the white and dilute brown parent-strains the reverse relation was noted. Furthermore, the differences between these age classes with which Little dealt were only slight.

More recently Bittner described another case of what he interpreted as linkage, namely, one between the factors determining transplantability of a certain tumor and the color of the skin; but in this case also, the differences in the percentage of takes in different groups of white mice differing in their hair color were slight. As to the effect of sex on transplantability of tumors, it is conceivable that sex hormones may, under exceptional conditions, favor the growth of certain mammary gland carcinomata in the same way as, in accordance with our previous observations, they may do in benign tumors of the mammary gland; however, such an action is not likely to affect fully developed carcinomas; they no longer respond, as a rule, to hormones. We, as well as Strong, Cloudman and Bittner, assumed that differences in the percentages of takes of tumors originating in the same animal, in hosts with a similar genetic constitution, depend upon differences in the characteristics of these tumors, but we do not agree in our interpretation as to the nature of such differences.

Before attempting to evaluate the results of these investigations, we may consider some earlier findings of a related nature. The study of multiple spontaneous tumors developing in the same individual was begun as early as 1907, when the writer, working with mice, noted that the structure of multiple carcinomata originating in the same animal was very similar, although not identical. At that time we suggested that it might be possible through transplantation of such tumors to determine whether the characteristic behavior of different carcinomata in strange hosts was due to factors inherent in the tumors or in the hosts. Woglom (1919), who had carried out such transplantations, found that the large majority of multiple tumors arising spontaneously in the same animal, behaved similarly after transplantation into the same strain of mice, but in a minority of cases differences did occur. Especially striking in this respect was the transplantation of three spontaneous tumors which had developed in the same mouse. One of these was readily transplantable into other mice, while the other two retrogressed following a temporary period of growth, and one tumor retrogressed more readily than the other. However, in these experiments Woglom wished to determine whether the behavior of tumors after transplantation depended upon adaptation of the tumors to the environment as it existed in the animal in which they had originated, or whether it depended upon the growth energy of the tumor at the time of transplantation. In the former case all the transplants should behave in a similar manner, since all these tumor cells had been reared in the same environment, while in the latter case the tumors should behave differently from one another because the growth energy is a variable factor, which, according to Bashford, differs at different times even in the same tumor.

The basic assumption underlying the interpretation of Strong and his collaborators is that the difference in the behavior of two tumors arising spontaneously in the same mouse is due to differences in the mutations of genes in somatic cells and, therefore, to the differences in the gene sets of these two tumors resulting from these mutations. But this, it seems, is not the only possible interpretation of this finding. We know that various normal and also embryonal tissues show different degrees of transplantability; thus, cartilage may be homoiotransplanted successfully in cases in which thyroid cannot, although both tissues can be autotransplanted equally well. These two tissues, when taken from the same individual, possess the same individuality differential but differ in the constitution of their organ and tissue differentials, and this latter difference may cause variations in their sensitiveness and transplantability. We also know that normal tissues differ much in the growth momentum which they possess; for instance, the normal and sensitized uterine mucosa may exhibit quite a different degree of proliferative activity after homoiotransplantation. Now, if we assume that during the transformation of normal tissues into cancerous tissues a *graded* increase in growth energy occurs and a concomitant change takes place also in the resistance to the injurious effects of transplantation, and if we furthermore assume that in two tumors, developing spontaneously in the same individual, this transformation has progressed to a different degree, then we could explain the ob-

servations of Strong without having recourse to the assumption of different genetic mutations in different somatic cells of the same individual.

However, there are certain other conditions which may correctly be attributed to genetic conditions. Thus Strong and Bittner observed in the course of their transplantations that in the closely inbred strain, "dilute brown," a change suddenly took place in the transplantability of a certain tumor and they attributed this change to a selection within the larger strain of a certain substrain or family, which thus evidently differed in genetic composition from that of the main strain. This agrees with our previous observations, in which we had found a similar change in the transplantability of tumors and also in the percentage of spontaneous tumors developing in a strain of mice, due to the splitting off of certain families possessing a somewhat different genetic composition.

From our findings after transplantations of normal tissues and of several tumors, we concluded that in a general way the outcome of transplantation depends upon the relation between the organismal differentials of host and transplant; and this means that it depends, essentially, upon the genetic constitution of the tumor cells as well as of the host cells, and that the reaction of the latter takes place in response to the strange genes carried by the transplant. In the strict sense the reaction does not, however, occur against the genes as such, but against certain physiological and biochemical mechanisms developing in transplant and host on a genetic basis. Strong expressed more recently the same idea that the result of tumor transplantation is a function of the genetic composition of both host and transplant. In this respect, then, the various investigators are in agreement.

There still remains to be discussed the relation between the hereditary predisposition to cancer and the change which takes place in normal cells during their transformation into cancer cells. These two conditions are distinct from each other. A comparison between the transplantation of normal tissues and of tumors which arise from normal tissues makes possible an analysis of the constitution of the individuality differentials of both, and we studied tumor transplantation largely from this point of view. Tyzzer, on the other hand, in common with Ehrlich, and also to some extent in common with Bashford, considered tumors as essentially different from ordinary tissues, as an abnormal condition which called forth an immunity peculiar to cancer, although certain exceptions to this rule were admitted. Thus Tyzzer assumed that the genetic study underlying transplantation of tumors might furnish an insight into the character of cancer and into the conditions which cause its development. Similarly, Little, Strong and Bittner infer a similar connection between the hereditary factors determining transplantability of tumors and the origin of tumors, and in this sense, Bittner intimates that the same dominant multiple factors which determine the transplantability of tumors may determine, also, the origin of cancer.

We shall now attempt to analyse still further the various data which we have discussed, and to determine, if possible, the significance of genes in the transplantation of tumors. For this purpose it will first be necessary to consider

again the principal factors which may enter into the transplantation of normal tissues and of tumors. There is (1) the relation between the individuality differentials or species differentials of host and transplant. This, as we have seen, plays a role in cancers as well as in normal tissues, numerous data confirming such a conclusion. (2) Variable factors relating to the mode of inoculation or transplantation may greatly influence the number of successful transplantations; these are non-genetic in nature. To mention an example: the usual figures for takes relate to experiments in which the transplant is placed into the subcutaneous tissue. Intracutaneous or intraperitoneal inoculation may alter these figures considerably. As we have seen, transplantation into the brain, and especially into the anterior chamber of the eye, may make possible a tumor growth, which would not take place after subcutaneous transplantation. And, as various investigators have shown, even heterogenous cancers may grow in the chorio-allantoic membrane or in the anterior chamber of the eye. Likewise, the amount of material inoculated is of importance. In many instances, transplantation of a larger quantity increases the number of successful transplantations. But if the quantity exceeds a certain optimum, the number of definite takes may decrease again in certain cases, because the added tissue may increase the amount of material which may serve as antigen and call forth the production of injurious immune substances. Also, in the case of mouse leukemia the quantity of injected leukemic cells helps to determine the result. While, as Furth has shown, a single leukemic cell may be able to transfer the new formation to another host, on the whole, prospects of a successful transplantation are greater and this tumor-like condition develops and kills the inoculated mouse more rapidly if the number of inoculated cells is greater. Also experimentally it is possible to diminish the virulence or growth energy of tumor cells by the application of various physical and chemical agents. (3) Certain extraneous, non-genetic factors which alter the susceptibility or the power of resistance of the host tissue to transplanted material. Application of X-rays, injection of colloidal dyes or other material, may diminish the resistance of the host, presumably by affecting the reticulo-endothelial system. There is good reason for assuming that also without the use of these experimental means, differences exist in different individuals and strains in the intensity of the reaction against transplants bearing a different organismal differential. (4) Differences in the resistance of different tissues, of which the tumors are composed, to injurious influences which may prevail in the host. These differences we have found in normal tissues, such as thyroid and cartilage, and there is evidence that they exist also in tumors. Thus the malignant chondroma of a mouse observed by Ehrlich could readily be transplanted into other mice, irrespective of their genetic constitution. The tumor grew slowly but was able to resist unfavorable conditions. When a piece of cartilage becomes permanently endowed with greater growth energy and thus assumes the characteristics of a tumor, it still retains some of the essential characteristics of cartilage, such as its relatively great resistance to the action of injurious bodyfluids and antagonistic cells. On the other hand, if thyroid becomes endowed with great growth potentiality, it likewise still

retains some of the essential characteristics of thyroid tissue and is, therefore, more susceptible to injurious influences. There is good reason for assuming that different tissues, such as cartilage and thyroid, and cancers derived from them which develop in the same host, possess the same genetic constitution and the same individuality differential, and that differences which such tissues and cancers show, are therefore, in all probability, directly non-genetic, although ultimately they depend also on the constitution of the germinal gene sets. (5) There are certain factors of an environmental nature which may also, under some conditions, influence the number of successful transplantations. Severe undernourishment may diminish it; hormones may affect the transplantability. Thus according to the recent experiments of Gross, transplantation of a mouse sarcoma succeeded more readily in sexually mature male than in female mice. He could make it very probable that the ovary gives off a substance, presumably a hormone, which had this inhibiting effect on the growth of the transplanted tumor. This is of importance, because it has been taken for granted by some investigators that slight differences in the effect of sex on the number of takes observed at a certain time of life were of genetic origin. However, it must be noted that Gross carried out intracutaneous transplantations and that under these conditions the existence of the tumors is a very labile and rather precarious one, in which slight interferences, which in transplants growing under more favorable conditions would hardly be noticeable, may affect quite definitely the fate of the tumor. The effect of the hormone in this case is presumably an indirect one. (6) Other intrinsic factors such as growth momentum, immunizing power, and adaptability of tissue to the condition of the host, all of which are greater in tumors than in normal tissues.

As far as the growth momentum is concerned, its constant increase in cancer tissue over that in the normal tissue from which it originated, is perhaps the most characteristic feature of tumor tissue. This increase in growth momentum makes it possible for cancer tissues to resist injurious influences to which normal tissues would succumb, the rapid cell multiplication probably increasing the ability of the transplant to absorb and neutralize injurious substances circulating in the host. Associated with this greater growth momentum there is usually a diminution in differentiation of the cancerous cells, which may likewise diminish the sensitiveness of the transplant to injurious factors under certain circumstances. However, the growth momentum is not a stationary condition; in a very large number of instances it has been observed that during the first transplantations of a tumor, whether a carcinoma or a sarcoma, the growth energy increases. Such an effect is typical, as we found about forty years ago in the course of our first transplantations of sarcoma of the thyroid gland in rats, and it has since been noted by many other investigators. There is no justification for assuming that so regular an occurrence, which does not depend upon a single tumor cell but may be noted after transplantation of various parts of the tumor, is due to a haphazard somatic mutation. It is presumably due to the stimulation exerted by incisions into the tumor and by the process of transplantation. A similar stimulation has been

noted also in the transfer of leukemic blood cells into other susceptible individuals. In the mammary gland, where the development of cancer out of normal tissue under the influence of hormones can be followed very well, it can be seen that, step by step, the growth energy of the tissue on which the hormone acts increases, and that as soon as a certain stage of intensity in this stimulation has been reached, the transition into abnormal growth takes place, provided the conditions transmitted by the germ cells make the gland tissue responsive to the action of the hormones. Furthermore, it is not a single cell which is altered, but more complex structural units, the acini and ducts of the mammary gland, undergo this cancerous change; and the latter does not depend upon the amount of newly formed tissue, but on the intensity of the growth stimulation which the gland structures have undergone. There is, thus, no indication that this process is caused by the occurrence of somatic mutations and that the cancer-producing stimuli in general are effective because the right kind of somatic mutations are produced. Although, therefore, the facts do not warrant the conclusion that this process of stimulation acts by way of the genes, on the other hand, the conditions which determine the degree of responsiveness of the tissues to the stimuli are transmitted by genes, but by genes of the germ cells and not of somatic cells. Eisen found that in the course of serial transplantations of a mammary carcinoma, which arose spontaneously in a rat belonging to a closely inbred strain, noticeable variations in the growth energy were lacking in the different generations of transplants; he attributes the constancy in the slow growth rate in the course of serial transplantations to the homozygous constitution of this strain and believes that when an increase in growth energy is noted in the course of the first generations of grafts, this is due to differences in the genetic constitution of different members of the strain. However, it can be shown that this increase in growth energy has in many cases been observed also in closely inbred strains. It is certain that this phenomenon is not due to selective processes in an impure strain of animals. But it is not observed in the case of all the tumors; to some extent, it seems to depend upon differences in the stability of the tumors used for serial transplantations.

As already stated, the primary condition required for the development of malignant tumors is an augmented growth momentum, and this augmentation may continue to take place in the course of transplantations of the cancerous tissue; it is one of the principal causes for the additional number of successful transplantations or "takes" which may take place during serial transplantation, and which accompanies the increase in growth momentum. But, omitting here from consideration, differences in the receptiveness of the host for the transplant, there are still other variable factors involved in the number of takes, which are situated in the tumor cells; among such factors we have referred above to differences in the resistance of the tissues to injurious conditions, which is likewise not directly genetic in character; and a third factor consists in the changes of an adaptive nature which can be seen sometimes after continued transplantation of tumors, changes which also occur after successive inoculations of bacteria and after longer continued exposure of

certain protozoa to various injurious conditions. In all these cases we may perhaps have to deal with alterations in cytoplasmic or nuclear-cytoplasmic mechanisms corresponding to the persisting modifications of Jollos ("Dauer modifikationen"). Such processes of adaptation have been observed under various circumstances; for instance, leukemic cells, which at first could be transferred only to X-rayed individuals belonging to an unfavorable strain of mice, could subsequently be transferred, also, to other individuals belonging to the unfavorable strain which had not previously been X-rayed. We shall discuss these processes of adaptation more fully in the next chapter.

In accordance with this interpretation of apparently spontaneous changes in growth momentum and takes which, as a rule, occur in the course of serial transplantations of cancerous tissues, we may likewise interpret the differences in growth momentum and transplantability which have been observed between spontaneous tumors originating in different mice of the same inbred strains, or even in the same mouse, and which we have already mentioned in this chapter. It should be expected that some differences may develop during the process of cancerization of normal tissues. This process may be somewhat farther advanced in some beginning tumors than in others, and there is no reason for attributing such differences to somatic mutations. Changes of the opposite kind take place during embryonal development; here, associated with a greater differentiation of the tissues, a gradual diminution in growth momentum and, correspondingly, in transplantability occurs; and these changes taking place during embryonal life are irreversible. But they are not due to a series of successive somatic mutations; nor should we be justified in attributing typical changes in growth and differentiation in the granulosa of follicles, previous to and during the process of maturation and corpus luteum formation, to a continuous series of somatic mutations. All these considerations make it improbable that either the transformation of normal tissues into cancers or the variations in growth momentum and transplantability of fully developed cancers are due to somatic mutations. However, as stated, the organismal differentials, and therefore also the genetic constitution of tumors, are important factors in their transplantability, as well as in the production of immunity against tumor transplants.

After transplantation of tumors, as well as after transplantation of embryonal tissues, processes of immunity can be more readily demonstrated in the host than after transplantation of normal tissues. We shall discuss these processes of immunity in tumor transplantation somewhat more in detail in a succeeding chapter. Here, it may be stated merely that the genes in the piece of tumor, which are strange to the host, are the precursors of those constituents of the organismal differentials in the tumor, which may function as antigens. The difference between the individuality and species differentials of host and transplant not only gives rise to the primary local defense reaction of the host against the tumor, but it also subsequently causes the transformation of these strange constituents into antigens and thus leads to the production of immunity. It is especially when a tumor, following a period of growth in a host, retrogresses that the host becomes immune against a second transplant

of the same kind of tumor, or of a tumor resembling the first one, as far as the constitution of their organismal differentials is concerned.

Gorer has observed that when a transplanted tumor has retrogressed, hemagglutinins appear in the blood of the host, which are directed against the erythrocytes of the donor of the tumor. He could show that when certain strains of mice and certain tumors were used, there were several kinds of hemagglutinogens present in the red corpuscles of the donor of the tumor which gave rise to the formation of hemagglutinins, and the number of these agglutinogens seemed to be approximately the same as the number of "susceptibility factors" for the growth of the tumors, as determined by the proportion of takes in the F_2 and backcross generations of hybrids between two strains varying in their susceptibility to the tumor, in accordance with the theory of Tyzzer and Little. He concludes, therefore, that it is the hemagglutinogens which represent the genetic factors needed for the growth of a transplanted tumor. Lumsden also noted in rats, in which tumors had retrogressed, the presence of hemagglutinins for the erythrocytes of the donor of the tumor. As we shall see later, it is unlikely that the number of factors needed for the growth of a transplanted tumor can be determined in a valid manner by the method mentioned. However, it is probable that differences in the constitution of the individuality or species differentials of cells and tissues in different individuals or species extend also to the erythrocytes, and that here they may be represented by agglutinogens, and that the constituents of the individuality and species differentials in the tumor cells, which are strange to the host, give rise to several kinds of immune substances, one of which consists of hemagglutinins. As stated, we believe that it is the genes of the tumor, which are not represented in the host, which are the precursors of strange constituents of the individuality and species differentials in the tumor, and which thus, indirectly acting through the organismal differentials, may give origin to processes of immunity and thus help to determine the fate of the transplanted tumor.

As to the determination of the "susceptibility factors" necessary for the growth of a tumor in a host by counting the number of takes in the F_2 hybrids between a favorable and an unfavorable strain, this is in principle the method which is used for establishing the number of multiple factors required for the appearance of a character in an individual, in case the father and mother strain differ in the number of the genes, needed for this purpose, which they contribute to the fertilized egg. In regard to the number of "susceptibility factors" found by using this method of determination, this differs in each kind of tumor; by making a sufficiently large number of assumptions as to the number of factors directly concerned and by having recourse to modifying factors, it will be possible, approximately, to fit all ratios found in the F_2 generation of hybrids into a certain formula; but it is difficult to see the advantages gained by establishing such a formula applying only to one particular tumor. However, the growth of a tumor depends not only upon certain genes in the host,, but, as we have already indicated, various factors of a primarily non-genetic nature help to determine the number of successful

transplantations in the F_2 generation of hybrids between susceptible and non-susceptible strains, such as the different degrees of resistance, the growth momentum, and the mode of inoculation of a tumor. The number of successful takes in the F_2 generation depends, therefore, not only on the relations of the organismal differentials of host and transplant, but also on various secondary factors, and the number of takes in F_2 hybrids might be quite different if these secondary conditions were altered. Moreover, if we use the percentage of takes of tumors as the criterion for the presence of genes in the tumor, which are compatible with those of the host, we apply a relatively coarse standard of measurement. There is no intergrade between take and non-take; the tumor either grows continuously after transplantation, or it does not grow; it may grow for a while, then retrogress and ultimately disappear. But there exist various finer kinds of distinction between degrees of compatibility or non-compatibility of host and graft, such as variations in growth energy, in the length of the latent period, and in the number of cells or size of a piece of tumor tissue which permits a successful transplantation. If these variables should also be considered, the gradations in the fate of tumor transplants in the F_2 generation would be much greater, and correspondingly, the number of factors which are supposed to determine the compatibility between host and transplant would likewise be found to be much greater. By taking merely into account the proportion of the number of takes to the number of non-takes in the F_2 generation, a threshold value is determined, namely, a slight excess or deficit in the sum of the large number of variables which decide the continuous growth or lack of growth of the transplanted tumor. Such a determination is not identical with the study of the differences between the individuality and species differentials of host and transplant.

In transplantation of normal tissues, much finer standards of measurement are used in the evaluation of the compatibility between host and transplant than in transplantation of tumors, where the percentage of successful transplantations alone is considered, and consequently the number of genetic factors on which this compatibility depends has been found to be great in the case of the former.

Although the non-genetic factors which we have discussed play a certain role in the transplantation of cancerous tissues, and, to a less extent, also of normal tissues, still, in both it is essentially the genes which fix the constitution of the organismal differentials, and therefore the result of transplantation depends largely on the relation of the individuality and species differentials in transplant and host. We have already referred to some of the essential facts which prove this conclusion. Autotransplantation succeeds as a rule, and autotransplantation means transfer under conditions in which the individuality differentials and their precursors, the genes, are the same in host and graft. Conditions might be different if somatic mutations were characteristic of tumors. In this case, an incompatibility might arise even between autotransplant and host; but this has not been observed. More or less approaching autotransplantation is transplantation into closely inbred strains; but it must again be stated very definitely that even the inbred mouse strains

D, C57 and others are not homozygous; not even the A strain, which approaches homozygosity more than other strains, is completely homozygous. But such inbred strains come near to this state to a degree which enables cancerous tissues to pass the threshold point which makes possible the continued growth of the transplants in other individuals of such a strain; whereas in individuals of different strains this threshold point has not yet been reached. On the other hand, normal tissues transplanted within these inbred strains quite frequently reveal the lack of a perfect identity between the individuality differentials of host and transplant. Analogous conditions are found if we compare transplantations of cancerous and of normal tissues from hybrids between two different inbred strains to parents, and vice versa, in cases in which the donors of the transplants belong to one of the two inbred strains. Tumor transplants, owing to the largely non-genetic characteristics which they have acquired, are able to pass the threshold point separating non-growth from growth in the new host, if parent tissue is grafted to the hybrids; whereas normal tissues, although they also do not evoke marked signs of incompatibility in the hybrids, still in many instances, call forth some reactions on the part of the host and may undergo a moderate degree of injury. However, in transplantations from hybrids to parents, tumors as a rule have not yet passed the point of threshold which allows them to grow, while normal tissues are injured in such a host to a higher degree than in the reciprocal transplantations, but because of the use of finer criteria the results are not considered, to the same degree, to be completely unfavorable or negative as they are when tumors are used in this type of transplantation. These differences between normal tissues and tumors are then not caused essentially by differences in the genetic constitution, but either by non-genetic factors, or by the methods applied in the evaluation of the results. If we deal with other strains which have not yet reached so high a degree of homozygosity, we should find various averages in the number of takes; the less the strain has been made homozygous by close inbreeding, the greater should be the number of animals which need to be examined in order to arrive at valid figures indicating the connection existing between compatibility of tumor and host and the genetic relationship between donor and host.

Additional data which prove the significance of the individuality and species differentials for the successful transplantation of tumors are furnished by experiments in immunization against tumor grafts. It is possible, although only to a limited degree, to immunize an animal by a previous transplantation of normal, and especially of embryonal tissue or by injection of red blood cells against a tumor transplant. In order to accomplish such an immunization against the individuality differential of a tumor, the tissue serving as antigen must belong to the same species as the tumor to be inoculated subsequently, but it must differ in the genes which determine individuality from those of the host. Such genes, which occur in the antigen but not in the host, give rise to the immune mechanism, and, in all probability, to the formation of antibodies. If antigen and host are heterogenous in their constitution, then the developing immune mechanisms are directed only against heterogenous, but not against

homoiogenous constituents of the antigens. Correspondingly, the immunity which is found in animals in which a homoiogenous tumor has retrogressed is directed only against the same or against related homoiogenous tumors.

In accordance with the theory that the organismal differentials are primarily responsible for the compatibility between tissues, we have assumed that it is the genes, or rather their derivatives, in the tumors which are strange to the host which call forth and determine the intensity of the reactions of the host against the transplant; and that the genes and their derivatives which are identical in host and transplant do not enter into these reactions, or do so only to a slight degree. We thus define in a more exact manner the cause of the reactions between host and graft. According to the terms of Mendelian heredity, the genes, which differ in host and transplant, are dominant over the genes which are identical, the latter being recessive, although as we have seen in our discussion of the transplantation of normal tissues, there is the possibility that also the latter may exert a certain effect.

In agreement with the interpretation given here are the results which Eisen and Woglom obtained in immunizing rats against the growth of a transplanted mammary gland adenocarcinoma, which had developed in a rat belonging to the inbred August strain; this strain was the offspring of a cross between two inbred strains (990 and 1561). The mammary gland tumor could be transplanted successfully into 100% of the August strain rats and into 78% of the 990 strain rats. Previous inoculation of embryo skin derived from August strain rats was not able to immunize August strain rats; nor was it possible to immunize 990 rats against the growth of the adenocarcinoma by means of strain 990 embryo skin; but embryo skin of August strain rats was very effective in immunizing animals belonging to strain 990. This is a good illustration of the fact that it is the strange genes which make possible the development of efficient antigens, and that it is the degree of strangeness of the individuality differentials in host and transplant which determines the degree of the antigenic effectiveness of the normal tissues or tumors.

Another difference between normal tissues and tumors or tumor-like tissues has been noted by Furth in his experiments concerning the transmission of leukemia in mice. He observed that in certain inbred strains a large proportion of the animals become affected by this disease. If leucocytes from a leukemic mouse were injected into other normal mice of this inbred strain, leukemia developed in all the animals, but leukemia could not be transferred to another strain in which spontaneous leukemia did not occur or was rare; it developed in 100% of F_1 hybrids between these two strains which had been inoculated with the leukemic cells. There was a decrease in transplantability in the F_2, and still more so in the F_3 generation. In backcrosses from hybrids F_1 to the susceptible parents leukemia developed in 100%, while in the backcrosses from F_1 hybrids to the non-susceptible parents it took in 50%. Leukemia arising in a hybrid F_1 could be transferred to all mice belonging to the susceptible parent strain; this is contrary to what should be expected according to the theory of the organismal differentials and to what is actually found in normal tissues and in mouse carcinoma. On the other hand, leu-

kemia could not be transferred to any individuals belonging to the non-susceptible parent strain. The results were variable in F_2 hybrids; leukemia could be transferred in 50% of F_2 hybrids.

These results could perhaps be explained if we assume that in addition to the gene sets derived from both parents, which determine the organismal differentials of these leukemic cells, there is present in the F_1 cells an intrinsic stimulus (Gi), derived from the parent which is susceptible to the development of spontaneous leukemia. The possession of this intrinsic stimulus converts the lymphoid cell into a leukemic cell and makes it possible for this cell to proliferate in an abnormal manner.

In the inbred strain of mice in which leukemia occurs spontaneously in a high percentage of cases, a factor (Ge) is present which stimulates or otherwise makes it possible for the leukemic cells to multiply and thus to transfer the disease, while in other strains this auxiliary factor is lacking. The activity of both the intrinsic factor within the leukemic cells and the auxiliary factor would enable the cells to overcome the resistance to the growth which is due to the presence of a combination of a set of strange genes and a set of genes identical with those of the host. We have referred to a similar condition already in the preceding chapter, when we discussed the effect of the continued action of Ge (hormones) on transplantation of not yet full cancerous tumors.

Further complications may be due to the fact that long-continued transplantations may modify the immunological characteristics of tumor cells and, according to MacDowell, also of leukemic cells. However, not all leukemic cells arising in F_1 hybrids of two strains, one of which has a high and the other a low incidence of spontaneous leukemia, behave in the manner observed by Furth and Barnes. Kirschbaum and L. C. Strong found that the leukemic cells from F_1 hybrids between the CBA and F strains behaved in the same way as typical carcinoma cells originating in such hybrids, while the leukemic cells from other kinds of F_1 hybrids in which leukemia had been produced experimentally could behave in an entirely different manner. But notwithstanding the existence of such complications which may arise, in general, it may be concluded that it is the organismal differentials in host and transplanted tumor and the genetic factors of which these differentials are the expression which primarily determine the fate of the transplant in a given host.

The genetic constitution of an individual influences the receptivity or resistance to the inoculation of a tumor by way of the individuality and species differentials of which the genes are the precursor elements. Furthermore, there are indications that special growth promoting substances may aid in the growth of transplanted cancer cells in certain cases, and it is probable that these growth promoting substances (Ge) which may be either hormone-like or virus-like, are also ultimately determined genetically. Organismal or individuality differential substances may be fixed in tissues as well as be present in the circulating bodyfluids. In certain respects they

represent gene hormones through which in the course of embryonal development gene effects may be transmitted to the recipient tissues and organs.

There are two experiments which indicate that substances of this kind may be transmitted by means of parabiosis from one animal which is genetically receptive to the growth of a transplanted tumor to the partner which is genetically resistant to such a tumor. Thus Zakrzewski observed that a Wistar rat, a strain not susceptible to the growth of the Jensen sarcoma, could be made susceptible by the parabiotic union with a susceptible Warsaw rat. Similarly Cloudman found that a hepatoma which originated in the C57 leaden strain, and which could readily be transplanted into mice belonging to this strain, but which could not be transplanted as a rule into black C57 strain mice could be made to grow in the C57 black if the latter was united by parabiosis with a C57 leaden strain mouse.

However, as a rule it does not seem to be possible to change the inherited strain receptivity or resistance of an animal by parabiotic union with an individual belonging to a strain differing in these respects from the first strain. Each of the two partners retains its own specific mode of reaction against the transplant. It is perhaps necessary that the differences in the constitution of the individuality differentials of the two parabiotic partners do not exceed a certain limit if a differential favorable to tumor growth shall exert its characteristic effects in the second partner. But as stated it is possible that in addition special substances favoring the growth of certain cancer cells may be involved in this effect.

Chapter 3

The Relation Between Growth Energy, Adaptive Processes and Organismal Differentials in the Transplantation of Tumors

In order to evaluate the role which organismal differentials play in the growth and transplantability of tumors, it will be necessary to consider separately certain variable factors which, in their interaction with organismal differentials, may influence the results of transplantation. Among these the most important ones are changes in growth energy and adaptive processes which may take place in the tumor in the course of serial transplantation in response to conditions present in the host; processes of immunity may also be considered as adaptive changes, but they occur in the host as a reaction to the growth of the tumor. Adaptive processes in the tumor may consist in changes in the readiness with which organismal (individuality) differential substances are produced and given off into the circulation of the host; likewise, the sensitiveness of the tumor and its power of resistance to injurious substances of the host may be modified; this would be added to primary differences in the sensitiveness which distinguish different types of tumors.

However, the degree of adaptability of a tumor to a new environment may be determined, in addition, by variations in growth energy which may take place in the course of serial transplantation. The growth energy of tumors was considered by us (1905) as one of the factors on which depends their transplantability, a low degree of growth energy rendering transplantation more difficult. In addition, we recognized in the host, as significant for the fate of the graft, a factor corresponding to what we later defined as individuality and species differentials. Among the growth factors we differentiated those inherent in the tumor cells (Gi) from others circulating in the bodyfluids of the host (Ge), and furthermore, we differentiated factors which permit a tumor to live, without necessarily enabling it to grow, from other conditions which enable it to grow. The growth energy was measured by the duration of the period of latency as well as by the rapidity of growth of the visible tumor. Different tumors were seen to differ very much in their growth energy and in their ability to withstand the injurious conditions associated with the process of transplantation, and among the latter there were some tumors which did not grow even after autotransplantation. We distinguished, therefore, between weakly and rapidly growing tumors, between temporarily and permanently growing tumors, and between transplantable and non-transplantable tumors; also between stable and labile tumors, the former retaining their growth energy unaltered, the latter, as a result of

various stimuli, showing an increase in growth energy, especially in the course of the first transplantations. If the constitutional factors in host or transplant, in particular the relations between organismal differentials of host and transplant, are unfavorable, the various kinds of growth stimuli may not be able to overcome the obstacles to transplantation, but on the other hand, if the inherent specific tumor stimulus (Gi) is very strong, the tumor may be able to overcome a not quite adequate constitutional condition and may dispense with growth hormones or other growth promoting substances (Ge) circulating in the bodyfluids. The less favorable the constitutional condition of the organismal differentials, the stronger must be the growth factors, those present in the host as well as those residing in the transplanted tumor cells, if a continuous growth of the tumor shall be accomplished.

Because of the action of these variables, including the relationship between the individuality differentials of host and transplant, growth energy and transplantability of a tumor do not need to follow a parallel course. The difference between these two sets of conditions was especially marked in the case of a carcinoma originating in a Japanese waltzing mouse studied by us; all the transplants grew, but the growth energy of the developing tumors was, at least in the first generation, not great. Here the constitutional factors, the individuality differentials, in the host and graft were well adapted to each other. The distinction between growth energy and transplantability was subsequently emphasized also by Apolant, and it still is useful at the present time.

I. *Changes in Growth Energy*

Of the two sets of factors, the constitutional factors residing in the host and those determining the growth energy of the tumor cells, the latter were more readily accessible to experimental analysis and the first attempts were therefore directed towards their modification by exposing the tumor cells to certain physical and chemical conditions. These experiments revealed the degree of what may be termed the elasticity of the tumor cells, their ability to recover from injury and to regain the growth energy which had been diminished by their exposure to injurious factors. Such reactions on the part of the tumor cells also represent adaptive processes; but they are temporary, not permanent adaptations. In the early period of experimental cancer investigations, the writer determined the intensity of heat required to cause the death of the rat sarcoma cells, and Jensen, independently, made similar determinations in mouse carcinoma. While the methods used in these two cases were different, the results were of the same kind. We found, also, the conditions under which certain chemical substances, such as glycerin and KCN, kill the tumor cells. It could be shown (1903) by the use of intermediate intensities in physical and chemical actions that between the full virulence and the death point of the tumor cells there exists an intermediate stage, in which the latter are still alive though growing with a much diminished energy. In certain cases, tumors grew temporarily; then the

growth ceased and a retrogression took place. It is therefore possible to diminish experimentally the growth energy of cancers. These results applied equally to sarcoma of the rat and to adenocarcinoma of the mouse. In some instances a very interesting phenomenon was observed; after heating the tumor in vitro for twenty to twenty-six minutes at 44°C, the growth energy of pieces, after transplantation into a living animal, decreased, but following this early period of slowed growth a certain degree of recovery set in. While usually this recovery was incomplete and the tumors which developed remained smaller than is normal for unheated tumors, in some instances the recovery was complete. However, in other cases the growth energy remained weak and at last the resulting tumors became stationary or retrogressed. This was especially noticeable after heating pieces for fifty-five to sixty minutes, when there was a great decrease in growth and recovery was rare. But even under these conditions recovery sometimes occurred and a period of more rapid growth followed. Some tumors showed what we called an oscillating growth, in which a weak growth or a stationary condition, or even an incomplete retrogression, was followed by a definite but slow growth, and this again by a cessation of growth and retrogression. On the whole, the effects of the intensity of heat on the latency period, the growth energy of the tumors, and the number of retrogressions took a parallel course.

Inasmuch as the change in growth energy of tumors produced by an injurious external agent could persist for a number of cell generations, it became of interest to determine whether repeated applications of heat, in successive transplantations, would lead to a summation of the injurious effects, or whether in the course of subsequent transplantations a recovery might still take place. There was noted such tendency of the tumor cells to recover from these injurious effects and this process seemed to be aided by an intervening transplantation into a new host. However, the restitution of the full growth energy in previously heated tumors was delayed after transplantation under these conditions. There occurs then, after all, in these cases, a summation of injuries caused by the heating and the process of transplantation, but this condition may be followed after some time by recovery. Such a recovery may also take place in tumors which have been injured by other means than heat; a heterotoxin injures the tumor transplanted into a strange species, but recovery may occur after return into the same species, as Ehrlich has shown. Chambers, Scott and Russ noted the injurious effect of the action of X-rays on rat sarcoma. In this case, also, a gradual recovery was seen after successive transplantations. And inasmuch as the process of transplantation as such is an injurious one, we may conclude that this faculty to recover from injurious effects is one of the conditions that makes possible the continued transplantation into successive generations of strange individuals of the same species. While thus in most instances a summation of the injuries caused by heat, leading to irreversible changes, does not take place in successive generations, but instead recovery follows, the opposite effect, a state of increased resistance to heating as a result of repeated exposures to higher temperatures, is likewise lacking.

If, following exposure to a sufficiently intense heat the tumor cells are injured, they may no longer be able to resist to the same degree as normal cancer, the activity of the connective tissue of the host, which thus begins to envelope the tumor with a fibrous capsule and to restrict its expansive growth. But following transplantation into a new host, a recovery of the tumor again may be accomplished and the tumor cells may now predominate over the stroma cells of the host. Furthermore, in accordance with the diminution in growth energy following the heating, we found that the number of cells undergoing mitotic division is distinctly diminished, although mitoses are not quite suspended; however, mitotic proliferation may occur, as we have shown formerly, even in retrogressing tumors. Lastly, we noted that as the result of the depression in growth energy following heating, certain reparative processes, which otherwise could take place in the tumor, are inhibited; thus the growth of active tumor cells into the central necrotic areas and the replacement of the latter by these cells are retarded. As these experiments show, we are able to produce through experimental interference, depressions in the growth energy of tumors, with or without subsequent complete recovery, or with only a temporary recovery. A similar diminution in growth energy, number of mitoses and oxygen intake, has been observed by Maus, Craig and Salter after transplantation of mouse sarcoma 180 into immunized mice; as a result of the immune processes, conditions injurious for the tumor cells had been created.

The experiments to which we have referred so far, concern sarcoma, but similar results can be obtained also in experiments with carcinoma. Thus, the writer and E. P. Corson-White observed that if the growth energy has been depressed, either through graded heating of the pieces of carcinoma preceding transplantation or through transplantation of the tumor into unfavorable strains of mice bearing a different strain differential, transplantation of the injured tumor cells into other more favorable mice might lead to the development of tumors which grew much more actively than the injured tumor which had been used for transplantation, although as a general rule the tumors developing under these conditions showed less growth energy than the average normal carcinoma No. IX. It was possible through continued serial transplantation of depressed tumors to raise still further their growth energy. In this manner, tumor tissue which otherwise would have perished, could be saved. But also in this series, as in the preceding one, grafted pieces of tumor failed to develop when once a certain stage of retrogression had been reached. Certain types of tumors which are presumably very sensitive to injury may therefore not respond to these procedures with a resumption of their growth energy. It seems, moreover, that in different types of tumors the inherent potential growth energy differs and the behavior of retrogressing or stationary tumors may depend also upon this factor. There is a constant balancing between the inherent growth energy and antagonistic factors, such as marked differences between the organismal differentials of host and transplant, or direct injury of the tumor caused by the graded application of heat or of certain chemicals, or, in some

cases, also by microorganisms. In principle, all these and still other more or less accidental factors act in a similar way. Thus we can understand that under certain circumstances these two sets of factors may approximately balance each other and thus the oscillating growth which we have described may be brought about.

However, not only a depression in growth energy of tumor cells, but also the opposite effect, can be obtained experimentally, namely, an increase in growth energy in cells which possess either a normal or a very low level of growth energy, or which may be retrogressing. Clowes and Baeslack observed that in not very virulent tumors the growth energy may be stimulated through a very mild exposure to heat; after subjecting tumor material for one hour to a temperature of 40°-41°C, they noted a certain stimulation. Michaelis also found such a stimulation under similar conditions, as well as after the use of very low concentrations of otherwise poisonous substances. But, previous to these experiments, in our early serial transplantations of rat sarcoma, we had produced stimulation in tumors in which, as a result of injurious factors, the growth energy had been lowered, leading to a stationary state or to retrogression. In a number of such tumors it was possible, by mechanical means, such as pulling a thread through the cancer, making an incision into it, or excising a piece of the tumor, to bring about a resumption of growth, which occurred in certain cases even when transplantation of a tumor nodule to a different place in the same animal had no or only a slight effect. But in another experiment, transplantation of a stationary tumor into a second animal led to a complete restoration of the growth energy of the tumor, which subsequently could be further transplanted into other animals. Such a stimulation was accomplished in stationary and retrogressing tumors only if mitotic activity was still present in the tumor cells; if this had ceased, the results were unsatisfactory. Thus it is seen that certain mechanical factors, such as incisions, extirpation of pieces of tumor, removal of pressure exerted by a fibrous capsule, or the process of transplantation, may stimulate growth energy; but it may also be that, in some transplantations, the transfer to hosts with strange individuality differentials may have had an additional growth-stimulating effect.

We have referred already to the increase in growth energy which occurs quite commonly after transplantation of spontaneous tumors into other individuals of the same species and strains; this was noticeable in our first transplantations of rat sarcoma; it was very definite also in our transplantations of a tumor which had developed spontaneously in a Japanese waltzing mouse, and which could be successfully transplanted into all other Japanese mice. Although in this instance the individuality differentials in tumor and waltzing mice serving as hosts were sufficiently similar to allow takes in 100% of the transplantations, still there was a marked increase in the growth energy of the grafts in the early generations. This may therefore be attributed to a stimulation of the tumor cells resulting from the process of transplantation, as such. Similar effects of transplantation were observable also in the subsequent transplantations of chicken sarcoma by Rous and Murphy. Here it

was apparent, furthermore, that the more actively the tumor grew, the greater was the number of individuals in which it took. Likewise, in the experiments of Chambers, Scott and Russ with a rat carcinoma which had been injured through radiation, there was a parallelism noticeable between the change in growth energy and number of takes. It was also found in the transplantation of leukemic cells into individuals of the strain in which the leukemia had originated.

There was, moreover, in our experiments a correlation between the growth energy of spontaneous tumors and their transplantability into other individuals, and Woglom, too, noted a parallelism between the number of successful transplantations of spontaneous tumors into other mice and the growth energy of these tumors. However, Woglom also observed that even very slow-growing tumors may yield a high percentage of takes, an observation which corresponds with our above mentioned experiments with the Japanese mouse, and which may be explained essentially by the great similarity of the organismal differentials of host and graft. But, in general, a tumor with greater growth energy will be better able to overcome the resistance which relatively unfavorable constellations of the individuality differentials present, than a tumor with a lesser growth energy. In addition to this factor, also variations in the resistance of various tumors to injurious conditions and in the rapidity with which organismal differential substances are produced by tumor and host may interfere with the proportionality between growth energy and transplantability.

The increase in growth energy which so often follows the first transplantation of a tumor is limited; it usually reaches a maximum in the first or in one of the following generations of tumors and from then on remains approximately constant. But, on the other hand, there can be no doubt as to the reality of this change and the great frequency of its occurrence. On the contrary, the rhythmic variations in growth and transplantability of tumors, which, as Bashford, Murray and Cramer assumed, takes place in successive generations of a transplanted tumor and which they attributed to conditions inherent in the tumor, were probably caused by changes in environmental factors affecting the growth energy of the tumor cells. Bashford believed, furthermore, that through selective transplantation, a tumor may be divided into substrains, which differ in certain characteristics and vary independently of each other in regard to growth rhythms. He held that a tumor represents a conglomeration of cells endowed with different characteristics. These rhythmic changes were not found by Fleisher in the case of carcinoma No. IX, nor by Bittner in his series of transplantations. Bittner holds that variations in the individuality differentials of the hosts, due to the use of mixed strains of animals, are responsible for these apparent rhythms.

II. *Adaptation of Tumor Cells to Environmental Conditions*

In addition to the factors mentioned, we have to consider some special adaptive changes which take place between transplant and host in the course of transplantations, as an occurrence which may influence the transplanta-

bility of tumors and complicate the analysis of the organismal differentials. However, it is necessary to distinguish from real adaptive processes a condition which may lead perhaps to similar changes, but is different. As a result of selective transplantation, lines of tumors, differing in certain characteristics, may be separated from the original tumor. By always selecting the most actively growing tumors for transplantation it was thought possible to separate from the tumors with ordinary growth energy and transplantability, a line which exceeded this average tendency. In these instances we would have to deal not with adaptive changes in the tumor—the characteristics of the tumor cells remaining the same throughout—but with a selection of certain types among several already in existence. In the case of true adaptation, on the other hand, actual changes in the characteristics of tumor cells would occur. Ehrlich used such a method of selection in order to obtain readily transplantable tumors. He compared this procedure with that employed in order to increase the virulence of bacteria, where, in serial inoculations of certain microorganisms into susceptible animals, the most virulent strain of bacteria was selected for each inoculation. However, Ehrlich believes that at the same time changes take place in the tumor cells in the course of transplantation.

In contradistinction to the increase in growth energy in successive generations of transplantations which we had observed, Ehrlich stressed the increase in percentage of takes in successive generations of transplanted tumors, and in accordance with his conception of athrepsia, as the main factor which determines the life and growth of cells, he explained the increase in transplantability in the course of serial transplantations as due to a new production of "nutriceptors" in tumor cells, which, according to his views, took place under the unfavorable conditions following transplantations into new hosts. Thus, the behavior of tumor cells was explained in the same way as the origin of strains of trypanosomes resistant to trypanicidal substances. Ehrlich operated therefore, essentially with one variable factor, namely, the difference in the ability of different cells to attract foodstuffs to themselves, and he assumed that a selection takes place in cells which differ in their power to respond to unfavorable conditions with changes in their nutriceptor apparatus. However, it would be difficult to explain on this basis the fact that the variations in growth energy and transplantability which do occur do not always take a parallel course; Ehrlich did not take into account the differences which exist in the individuality differentials of different hosts, and he also failed to consider the effects of separating strains of hosts. Furthermore, he did not consider the relations which exist between growth energy and transplantability.

Various observations make it very probable that adaptive changes in the constitution of tumors can actually take place. The considerable increase in the number of takes, which has been noted by different investigators in the course of serial transplantation of spontaneous tumors into strains of animals in which they at first grew only with difficulty, is probably at least partly due to certain adaptive changes which have arisen in the tumors in the new host.

Thus Bashford and Murray found that the Jensen mouse carcinoma, which grew readily in Danish mice, but only with difficulty in English mice, began to grow at last also in the latter in the course of continued transplantations. Similarly, the first Rous chicken sarcoma which, according to Rous and Murphy, at first took only in blood relatives of the animal in which it had originated, after further transplantation grew well also in non-related chickens of the same variety, and after still further propagation it became adapted even to growth in different varieties of fowl. On the other hand, the second Rous sarcoma, an osteochondroma, grew from the start in all varieties of fowl, in conformity perhaps with the relatively low degree of sensitiveness of cartilage to differences in individuality differentials. Rous and Murphy observed also a selective process, which led to the opposite effect; by selecting weakly growing tumors for further transplantation, a line of tumors was propagated which tended to undergo retrogression. In this case evidently the tumor cells had been injured through unfavorable organismal differentials of the host, or through certain secondary factors—an injury similar to that obtained by heating—and after successive transplantations these injuries accumulated. We have already referred to the experiments of Duran-Reynals, in which marked adaptive changes were observed in Rous chicken sarcoma cells after transplantation into ducks; these changes affected primarily the agent situated in the cells, but secondarily, the cells themselves seemed to undergo corresponding adaptive changes, presumably under the influence of the agent they contained.

Similar in certain respects were the adaptations which Roffo noted in a transplantable rat tumor. Through continuous selective tranplantation he succeeded in adapting this tumor to growth in different varieties of rats. At last it could be successfully transplanted in 70 per cent of wild rats, in which it had not been able to grow at all in the beginning. It was of interest that also in these experiments there was a parallelism between the increase in transplantability and growth energy of the tumors, indicating that we may not have had to deal solely with an increase in transplantability due to special adaptive processes, but also to an increase in growth energy. Similar adaptive changes were apparently observed by Gheorgiu when he transplanted mouse tumors into very young rats. With successive passages the process of complete retrogression in the heterogenous animals became more and more delayed, until at last growth extended as long as to the twenty-seventh day following transplantation. After several passages in newly-born rats, in which presumably the mechanisms of reaction against strange organismal differentials are not yet fully developed, the tumors could be transplanted also into older sucklings, but here the tumor did not live as long as in the very young animals. The retrogression and absorption in these young animals seemed to follow without the aid of leucocytes (lymphocytes). After reinoculation into mice the tumors grew with increased intensity. There are still additional experiences which point to adaptations taking place in tumors in the course of serial transplantations and causing an increase in their transplantability. Furth and others observed that also leukemic cells after continued serial transplantations be-

came more virulent, which means that they multiplied more rapidly in the host; at the same time, certain structural and other changes occured and these cells acquired the ability to propagate in alien strains into which they could not be transplanted in the beginning. However, in such experiments it is difficult to determine how far the increase in transplantability of the leukemic cells is due to the increase in growth momentum and how far it is due to actual adaptive processes to strange individuality differentials. In some cases, on the other hand, the contrary effect, namely, a greater sensitiveness to strange individuality differentials, has been observed in the course of continued transfers.

We have referred above to the experiments of Gheorgiu, in which adaptive processes, arising in mouse tumors, gradually increased the ability of these tumors to grow also in heterogenous, although nearly related species. Similar observations have been made in the Putnoky experiments, in which a mouse carcinoma could be serially transplanted into rats; but these we have discussed in an earlier chapter. We may, however, add here that in the early passages there was more necrosis than in later ones, in which the tumors were able to maintain themselves also in somewhat older rats. There was, moreover, a diminution in the amount of stroma in the rat-adapted mouse tumors. In these heterotransplantations, as well as in the transplantation of leukemic cells into different strains, certain structural changes took place in the course of continued transplantations; furthermore, rat-adapted tumors, when transplanted back to mice, showed a marked growth energy.

In a considerable number of experiments it was possible to make tumor or leukemic cells grow in unfavorable strains, if the aggressive power of the hosts had first been depressed by some experimental means. Preliminary treatment of the host animals with X-ray or with trypan blue had this effect. Cancerous or leukemic cells, which had been propagated for some time in such specially prepared hosts, were afterwards able to propagate in otherwise unsuitable strains, even without a preceding experimental depression of the aggressive power of the host animals. Another method, which led to similar results, was used by Margaret R. Lewis, who inoculated mouse sarcoma into mice belonging to strains which were genetically unsuitable for the growth of this tumor. The first inoculations of this kind were unsuccessful; but after repeated inoculation of pieces of this sarcoma into the same individual mice, the sarcoma grew in the end, and after the tumors had once succeeded in growing in alien strains they could be further propagated in these strains without much difficulty. Several investigators have found that tumors which did not grow after subcutaneous, intramuscular or intraperitoneal transplantations, grew successfully in the brain or in the anterior chamber of the eye. Such tumors could subsequently be successfully transplanted, also, by subcutaneous or intramuscular inoculation into animals in which originally they would not have grown in these places. But such an increase in the capacity of tumors to grow elsewhere after they had first been transplanted into the anterior chamber of the eye was not noted in some recent experiments which Greene carried out with rabbit tumors.

As to the mechanism underlying these adaptive changes, it might be assumed

that these are due to somatic mutations in the tumor cells, rendering the organismal differentials of tumor and host organisms more similar; thus the differential substances in the tumor, which act as toxins for the host, would be diminished, and the reaction of the latter, causing an injury to the tumor, would be lessened or prevented. According to this interpretation, mutations would make the tumor better able to resist injurious conditions. Thus Warner and Reinhard interpreted certain changes which they recently observed in tumors following treatment with X-rays as due to somatic mutations. They exposed two spontaneous adenocarcinomas, which originated in the dba strain and in the New Buffalo strain of mice, to 100 Roentgen units in vivo, or to 50 Roentgen units in vitro. The non-radiated tumors grew in 100% of its own strains, but not in strange strains. After radiation the tumors continued to grow in 100% of the mice belonging to the strain in which the tumors originated, but they now grew, also, in about 40% of mice belonging to strange strains. They concluded that this result was due to somatic mutations in the tumor cells. This is, however, improbable, because the genetic change, which would have been required to produce the adaptation of the tumor to the strange strain, should have lowered the successful transplantations to its own strain. Moreover, it does not appear likely that a random somatic mutation which had such an effect, producing the same percentage of takes in strange strains, should have occurred independently in two different tumors belonging to two different strains. Lastly, the Roentgen dose necessary for inducing mutations in germ cells is much greater than the one used in these experiments. It appears more probable that the X-rays affected a cytoplasmic mechanism, which caused, perhaps, a diminution in the production of the organismal (individuality) differentials in the tumor, and which therefore elicited a less active reaction of the strange strain against the transplant; this cytoplasmic change was then transmitted to successive generations of tumor cells. In general, the same objeections which can be raised against the opinion that cancers arise as the result of somatic mutations in normal cells, or that variations in the growth energy and in the number of takes, which occur in the course of the first transplantations, have such an origin, apply also to the assumption that adaptive changes are due to somatic mutations. The adaptive changes, consisting in an increase in growth momentum, and the gradual increase in takes in at first unfavorable hosts are again due, in all probability, to changes in cellular metabolism which are independent of somatic mutations. There is no indication that noticeable changes in the constitution of the organismal differentials are concerned in these adaptive processes.

This applies also to the Ehrlich-Putnoky carcinoma, to which we have referred previously. The behavior of this tumor suggests that no definite change in the species differentials of the tumor has taken place as the result of the serial transplantation of tumor cells into rats. This is true although the rat-adapted strain induces in the rats, in which it has grown and subsequently regressed, immunity against Walker rat carcinoma and Jensen rat sarcoma; but the mouse-adapted Putnoky tumor also has some immunizing effect, although it is less effective in this respect. However, it is significant that this

rat-adapted mouse carcinoma could not be transplanted in rats previously treated with normal or cancerous mouse tissues, while a previous treatment of rats with rat tissue did not prevent transplantation. We may therefore assume that the rat-adapted strain of the Putnoky mouse tumor bears essentially the species differentials of the mouse and it is possible that its apparently increased effectiveness in the production of immunity in rats may be due to its growth momentum, which is greater in the rat-adapted strain than in the mouse-adapted strain. Yet, even if the increase in immunizing power which distinguishes the rat-adapted strain from the mouse-adapted strain should not be due to the increased growth momentum, it still would not be necessary to attribute such changes to somatic gene mutations; instead, it might be attributed with greater justification to metabolic changes taking place in the tumor cells, independently of constitutional modifications of the organismal differentials. The same considerations apply to the alterations in the specific immunizing action which, according to MacDowell and his associates, leukemic cells undergo in the course of serial inoculation; properties are acquired which make these propagated lines of leukemic cells different in various aspects from the original leukemic cells from which they were derived; and similar observations have been made by Dmochowsky in the case of ordinary cancerous tissues.

Somewhat related to the experiments with the Putnoky tumor are those of Lumsden, which also indicate that a certain adaptation may take place between a tumor and a heterogenous host of a nearly related species, as indicated by the reaction of the tumor cells in tissue cultures. Lumsden finds that if a mouse carcinoma has been developing in a rat for a week, pieces of this tumor growing in vitro are not injured by the serum of the rat which was the host of the tumor, and in which, therefore, immune bodies have developed against the mouse carcinoma cells; but such a serum rapidly kills mouse carcinoma cells which have previously been growing in a mouse. Likewise, serum of a rat in which a rat sarcoma has grown is not injurious to mouse tumor cells which have been growing previously in a rat, but it is injurious to mouse carcinoma cells which have been growing in a mouse. Yet such tumor cells, which have become resistant to the effects of heterogenous immune serum acting in vitro, retain their specific sensitiveness to transplantation into a heterogenous organism. A mouse tumor is injured after transplantation into a rat, even if it has been growing previously in a rat. Lumsden assumes, therefore, that the immunity thus acquired by the tumor cells is active only against constituents of the blood and, moreover, that the tumor cells growing in a heterogenous host acquire the ability to use the amino-acids specific for the latter as building stones for proteins, which are no longer characteristic of their own but of the foreign species; this would imply that the species differential of the tumor cells changes into that of the foreign species. However, all the data known so far point to the conclusion that the animal organism transforms amino-acids into protein of its own kind. The adaptation occurring in the tumors growing in heterogenous hosts must therefore be due to processes of a different nature.

There have thus been established certain variable factors which affect the

transplantability of tumors into different kinds of hosts, such as the growth energy of tumors and their power to adapt themselves to conditions present in the hosts. These characteristics, or the potentiality to develop them, were acquired during the process of the transformation of normal tissue cells into cancer cells, and this transformation is due to the interaction of genetic factors, transmitted by the germ cells, with variable stimulating factors; both these sets of factors, the intrinsic genetic and the extrinsic stimulating ones, are active in the organism in which the transformation to cancer occurs. Such a process is a graded one, which takes place step by step, and it is probable that to the stage which has been reached in this transformation there correspond different degrees of those characteristics which distinguish tumors from normal tissues. Prominent among these characteristics is the increase in growth momentum and the range of variations which the growth momentum may undergo, and it is probable also that the ability to undergo adaptive changes was acquired, or at least intensified, during the cancerous transformation.

That adaptive changes to conditions otherwise injurious may be effected in tumor cells has been shown in a more direct way in experiments by Fleisher and the writer. We observed that intravenous injections of solutions of colloidal copper into mice diminish the growth-rapidity of a mammary gland carcinoma in these animals; but if tumors that have been subjected to the influence of colloidal copper for some time, are then transplanted into other mice which subsequently were injected with solutions of this substance, the developing tumors were found to be more resistant to the action of colloidal copper than a line of transplanted carcinomas which had not previously been treated in this way. Similar effects were noted when hirudin was substituted for colloidal copper. Both of these substances immunized the tumor cells in a specific manner. A corresponding decrease in the effectiveness of these tumor growth-inhibiting agencies could be observed if mice bearing adenocarcinoma No. IX were injected with either of these two substances from the second to the sixth day following transplantation, and again from the ninth to the thirteenth day; the effect of the second series of injections was diminished as the result of the immunizing influence of the early injections. This immunization affected the bearer of the tumor as well as the tumor itself. Likewise, some more recent experiments of Lignac suggest that the cells of a mouse sarcoma may adapt themselves to the action of trypan blue injected into mice; here, also, it seems that we have to deal with an immunization of tumor cells. Apparently, then, tumor cells may behave in a similar manner to trypanosomes, which also may become adapted to various injurious substances, such as trypanicidal preparations of arsenic.

We may then conclude that the increase which in many instances takes place in the growth energy and in the number of growing tumors following transplantation of spontaneous tumors, is due to different factors which have to be kept distinct. In the first place, the process of transplantation as such produces an augmented growth energy; this may be due to mechanical stimulations similar to those which induce regenerative growth. In addition there may, under certain conditions, come into play perhaps a direct stimulating effect of

a strange individuality differential. This increase in growth energy, other factors being equal, must lead to an increase in the number of developing tumors, and indeed, under these conditions there can be observed a parallelism between increase in growth energy and number of takes. Secondly, there may take place in the tumor more specific changes of an adaptive character; the strange individuality differential of the host seems to alter the tumor in such a way that it becomes less sensitive to the injurious action of the strange differential. In diminishing the injurious effects of the host this change itself also may, under certain circumstances, secondarily cause an increase in the growth energy of the transplant. Whether a strange differential will act merely injuriously on a tumor, whether it will also have a stimulating effect, or whether, in the end, it will produce adaptive changes, depends presumably upon quantitative relations between the degree of strangeness of the individuality differentials and the inherited power of resistance and other inherited characteristics, such as a certain modifiability of the tumor cells.

It may then be stated that transplantability, as judged by the number of takes of a tumor, is contingent largely on the relation between the organismal differentials in host and transplant, and on the ability of the tumor cells to withstand injurious influences of not well suited organismal differentials. The latter factor depends, among other conditions, also on the actual or potential growth energy of a tumor and on the ability of the tumor cells to undergo adaptive changes in different environments. The organismal differentials in host and tumor are determined directly by their genetic constitution, but the range of the potentiality of adaptation, the increased growth momentum, and the ability to undergo variations in growth energy are only indirectly determined by genetic factors transmitted by the germ cells; directly, they are determined by environmental factors which are active during the transformation of normal into cancerous tissues.

This relatively high degree of adaptability to different environmental conditions which we observed in tumors, distinguishes them from normal tissues, in which such an adaptive, plastic character of the cells cannot be demonstrated. For instance, attempts to overcome the action of unfavorable individuality differentials in the host by serial transplantation of normal tissues did not succeed. Thus, in our serial transplantations of epidermis the transplants soon died and while we found that cartilage cells could be transplanted serially and live for a long time—much longer than the animal in which this tissue originated—in the end they also died and the serial transplantation ended. It is possible that this difference between normal and tumor tissues is due to the difference in the growth energy which exists between these types of tissues. The greater growth energy which cancers possess makes it possible for them to resist difficulties which would destroy normal tissues, and gives the former a chance to react to a new environment with adaptive changes. But there is also the probability that the changes which take place in normal tissues when they are transformed into tumor tissues, introduce at the same time a new type of adaptability to strange organismal differentials, which normal cells do not yet possess; this power of adaptation would then represent a newly

acquired characteristic of tumors which distinguishes them from normal tissues.

However, there are some indications that also in normal tissues of higher organisms some processes of adaptation may take place. If we stimulate the thyroid gland of the guinea pig by means of iodine or anterior hypophyseal extracts, the stimulating effect ceases after some time and at last, after continued applications of these substances, a refractory state with less than the normal reactivity ensues. It is probable that in this case adaptive changes which occur in the cells exposed to such stimulating substances are responsible, at least in part, for the condition of tolerance attained. Similarly, if a piece of homoiotransplanted cartilage is left for a long time in the host, the reactions on the part of the host tissue against the transplant, instead of increasing or showing a cumulative effect with increasing length of time, seem, as a rule, to diminish in intensity. But, here, it is not certain how far the diminution in reaction is due to adaptive changes in the host or in the transplant.

Reference has also been made in an earlier chapter to the observations of Rhoda Erdman and Gassul, that a gradual adaptation of anuran amphibian skin to heterogenous amphibian anuran hosts may be accomplished by cultivating the former for some time in vitro in culture media, which were rendered more unsuitable through step-by-step addition of the foreign plasma from the species to which it was desired to adapt the skin. But in these experiments it is not certain that adaptive changes had actually taken place in the transplanted tissue.

Somewhat related investigations were carried out subsequently by Kimura, who cultivated chicken tissue in vitro in duck plasma and tissue extract. The chicken tissue thus prepared was used as antigen for the production of precipitins. These precipitins reacted with duck instead of with chicken antigens. Kimura concluded therefore that chicken tissue had assumed the characteristics of duck tissue as a result of adaptive changes taking place in the new heterogenous environment. However, instead of assuming so fargoing a change in the species differential of the chicken tissue within a relatively short period, the possibility may be considered that some duck plasma was admixed to the chicken tissue serving as antigen and that the adhering duck plasma was responsible for the production of the precipitins. That such a transformation of the individuality differential does not actually take place is also indicated by an experiment of A. Fischer, in which he showed that rat fibroblasts, which had been cultivated for more then twenty-three years in chicken plasma, still remained rat cells; they retained their species differential and cytotoxic immune serum directed against rat tissues injured the rat cells that had previously grown in chicken plasma in the same specific way as it injured fresh rat cells. In both the case of tumor tissues and of normal tissues we arrive, therefore, at the conclusion that in all probability definite changes in the species differential do not take place in the course of serial transplantation, and that the adaptive changes occurring in transplanted tumors under certain conditions are not due to somatic mutations.

In the experiments which we have discussed so far, an adaptation of tumor

cells to a different type of host was produced experimentally, or in other cases, the reactivity of the host against the cancerous transplants was diminished through injection of substances which in all probability inactivated the reticulo-endothelial system of the host. We have also referred to experiments in which the reactions of the host against transplanted cartilage became weaker in the course of time, thus indicating possible processes of adaptation which took place in the host under the influence of the transplant. Quite recently, Cloudman has published some experiments which point perhaps in the same direction. He found that an osteogenic sarcoma, which had originated in the tail of a C57 mouse and grew in 100 per cent of C57 mice inoculated with this tumor but grew in a much smaller percentage in D mice, took in a somewhat larger percentage of D mice which had been transferred at the beginning of their embryonal development into the uterus of C57 mice and had undergone further developments here instead of in the uterus of their real mother. While this treatment increased the number of successful inoculations and also the rapidity of growth of the transplanted tumors, the growth of the sarcoma was not decreased thereby in C57 mice which had developed in the uterus of D mice. Corresponding results were obtained in experiments with a malignant melanoma which had originated in the tail of a D mouse, and perhaps also in experiments in which Law increased by means of foster-nursing the number of successful transplantations of leukemic cells in mice belonging to a subline of the D strain, which differed from the one in which the leukemia had originated and which was less favorable for the transplantation of the leukemic cells possessing a different individuality differential. It is possible that the transfer of substances possessing a different individuality differential by way of the uterus or by way of the milk of the mother caused an adaptation of the host against these substances, which was thus rendered more tolerant against the strange individuality differential of the tumor cells. But it is also possible that the substance thus transferred into the mice serving as hosts supplied the latter with a carrier of the individuality differential more closely related to that of the tumor cells which the latter needed for a successful growth, or that substances introduced into the future hosts by way of the uterus or with the milk of the nursing mother supplied the hosts with an agent which stimulated the growth of the subsequently transplanted cells. There are indications that the effect observed in these experiments is only a temporary one; mice which were inoculated with the tumor several months after they had received the strange substance no longer reacted favorably to the transplanted tumor. However, it is not yet certain whether this loss of tolerance was due to the older age of the mice under these conditions, or whether it was due to the fact that the strange substance was gradually eliminated. All these experiments taken together do not, therefore, suggest that variations in the growth energy or in the percentage of successful transplantations of a tumor are due to changes in the organismal differentials in the host or in the transplant; but they point to the presence of factors, which, when added to the action of these differentials, may modify the mode of the reaction of the host against the transplant.

In comparing the conditions which influence the transplantation of normal tissues and of tumor tissues, we conclude that in both of these processes the relations between organismal differentials of host and transplant play a similar role, but that various factors of a secondary nature may obscure the significance of the organismal differentials, and this applies particularly to tumor growth. The conditions determining the growth of transplanted tumors include the factors which control the growth of normal tissues, as well as other factors which are specific for tumor tissue, such as the intensified growth momentum, the possibility of increasing this growth momentum still further, and the potentiality to undergo special adaptations in the course of serial transplantations. In the analysis of tumor growth and tumor transplantations, it is necessary to separate these various factors as much as is possible at the present time.

Chapter 4

Immunity and Organismal Differentials in Tumor Transplantation

In the preceding chapters we have analyzed the relation between the transplantability of tumors and the genetic constitution of the organisms in which the tumors originated, as well as of the hosts, and the individuality and species differentials of these organisms. It has been stated already that also immunity against cancer grafts may be an expression of the organismal differentials and from this point of view various aspects of this type of immunity will now be considered. We shall study, therefore, mainly those phenomena in immunity which have a bearing on the role which organismal differentials play in tumor growth, in particular, the constituents of strange organismal differentials which may readily function as antigens and thus induce immunity against grafted tumors.

Early investigators in this field, Jensen, Ehrlich and Apolant, Bashford and Murray, applied the principles established in the study of immunity against microorganisms, animal cells and proteins to the study of immunity against transplanted cancer. At an early stage of these investigations, a natural immunity and an acquired immunity to microorganisms and their toxins were distinguished. By natural immunity is understood a preformed constitutional resistance. The development of an active immunity, on the other hand, presupposes a previous interaction between the host organism and the strange cells or substances against which the immunity is acquired. In active immunity, substances (immune substances, antibodies) may be produced, which circulate in the bodyfluids of the host and tend to injure the strange cells or to neutralize those substances (antigens) which elicited the immune reaction. By injecting these bodyfluids of the actively immunized animals into other animals it is possible to transfer the immunity to the latter, which thus acquires a passive immunity. We have already discussed some of the conditions on which depends the existence or lack of natural immunity to the growth of transplanted tumors, namely, the relations between the constitutional genetic factors in the tumors, which are to be transplanted, and in the hosts, into which they are to be transferred; the latter may be individuals of the same strain or species in which the tumors originated, or individuals of different strains or species. The resistance to homoiogenous or heterogenous transplantation may thus be considered primarily as a manifestation of natural immunity, which depends on the relation between the individuality and species differentials of the host and transplant. Especially striking in this connection are the differences between the results of auto- and homoiotransplantation. Here, reference may again be made to the experiment in which Fleisher and the writer showed that the immunity

which becomes manifest after extirpation of a homoiogenous tumor does not affect an autogenous tumor growing at the same time in the bearer of the homoiogenous tumor, nor is the extirpation of an autogenous tumor followed by immunity against inoculation with a homoiogenous tumor.

The observation of the writer and of Jensen, that in animals in which a first inoculation of a homoiogenous piece of tumor was not followed by tumor formation, a second inoculation of a homoiogenous piece was also unsuccessful, suggested to Jensen the idea that as a result of the first inoculation immune bodies developed in the animal, which protected it against a second inoculation, and that the phenomena apparently attributable to natural immunity did in reality represent an acquired immunity. Subsequently, it was observed however that under the conditions of Jensen's experiments immune bodies cannot be demonstrated in the blood of the inoculated animal. Jensen's work was the starting point for the investigations of Ehrlich and Bashford, and their collaborators. Ehrlich and Apolant, extending to natural immunity against transplanted tumors their conception of natural immunity against microorganisms, assumed that specific X substances are needed to allow, in a certain host, the growth of bacteria as well as of tumor cells. If there is an insufficient amount of such an X substance present, a state of athrepsia exists in the host as far as the microorganisms or cancer cells are concerned and they are therefore prevented from growing in this host. Other investigators have attributed the natural immunity against transplanted tumors to the action of lymphocytes, and this factor they held responsible also for the development of an active acquired immunity against canter. Thus, in the case of the Rous chicken sarcoma it was observed that in naturally immune fowl lymphocytes collected around the tumor transplant; it resembled in this respect transplanted normal tissue, where likewise lymphocytes play a significant role.

When it was found that it is possible in a certain percentage of animals, which varies in different cases in accordance with the kind of tumor or host used, to produce an active immunity through inoculation of normal tissues or of certain kinds of tumor tissue, the view was expressed by Russell that all natural immunity against tumor grafts is in reality a manifestation of active immunity, due to the absorption of a certain amount of the inoculated piece, which thus acts at the same time as an antigen. Whether an animal proved to be naturally resistant (immune) or not depended therefore upon its ability to develop an active immunity. This conclusion of Russell, which represents an extension of Jensen's view, was very widely accepted and has found expression even in recent literature. However, while active immunity undoubtedly plays an important role in determining the fate of transplanted tumors, this interpretation does not explain why certain individuals should develop an active immunity, whereas others are not able to do so, and this is the important point which needs to be elucidated. In the case of normal tissues we have seen that such an interpretation would be inadequate. Here, the primary relation between the organismal differentials of host and transplant is the determining factor, and tumor tissue has retained in many essential respects the characteristics of normal tissues, with the addition of certain

peculiarities secondarily acquired. Also, in the case of transplanted tumors an active immunity develops only if there exists a primary incompatibility of the organismal differentials of host and transplant, although such a primary incompatibility between the organismal differentials of host and transplant may in certain cases be insufficient to prevent the growth of implanted tumors. But, as we have stated already, with tumors an active immunity seems to be of much greater importance in preventing the growth of the transplant than with normal tissues.

In the majority of cases it seems to be the strange organismal differentials, and in particular the strange individuality differentials, which serve as antigens in the production of an active immunity. Therefore, under normal conditions no immunity develops in mammals against autogenous spontaneous tumors; they are not antigenic. Conversely, because autogenous tumor tissue does not elicit immunity against itself in the bearer of the tumor, it may be assumed that the tumor tissue has essentially the same individuality differential as the other cells of the same organism. However, it has been shown that avian sarcomata and related tumors, produced by means of injections of tumor filtrates, may give origin to antibodies which are active against the autogenous tumor cells; but these antibodies are directed against the agent and not against the tumor cells. Furthermore, there has accumulated more recently some evidence which proves that in mammalian tumor tissue there may be present in addition to the organismal differentials, some antigens which are specific for a certain kind of tumor and not for the corresponding normal tissue, and perhaps others which are common to many different types of cancer. In these cases, special substances may serve as antigens.

There has been a certain reluctance on the part of some investigators, especially Bashford and his associates, to apply the term "immunity" to the mechanisms underlying the reactions against tumors developing in animals inoculated with the latter. They preferred the term "resistance," because in the course of time they began to doubt that a typical immunity, comparable to antibacterial immunity, develops at all against cancer cells. This doubt was based on the impossibility of demonstrating immune substances in the host inoculated with homoiogenous tumors and of transferring antibodies to other animals, which thus would be protected against the growth of a second homoiogenous tumor. However, this difficulty has disappeared in recent years; since it has become possible in various ways to demonstrate that such protective substances are formed. We therefore need not hesitate to consider these reactions against tumor grafts as evidence of an active immunity. The processes of active immunity are of special importance as far as the reactions against homoiogenous tumors are concerned. In heterogenous tumors the primary incompatibilities between host and graft become so strong, particularly with increasing distance between the species of the host and the bearer, that preformed processes may be sufficient to injure and kill the transplants.

An active immunity against inoculated tumors may be obtained under the following conditions: (1) When a transplanted tumor grows in an animal; the developing immunity is known as "concomitant immunity"; (2) in certain cases following the extirpation of a growing homoiogenous tumor; here

an active immunity which had not been demonstrated previously may become manifest, but there is reason for assuming that it was actually present already while the tumor was growing in the host; (3) after regression of a homoiogenous or heterogenous tumor, when an animal as a rule is found to be immune to a second inoculation of the same or of a similar kind of tumor; (4) after inoculation of normal tissues or of pieces of tumor unable to give rise to the formation of tumors; to a certain extent, animals thus treated are immune to the growth of a piece of tumor subsequently inoculated. We shall now describe the essential characteristics of each of these types of active acquired immunity, and shall also discuss (5) the presence of immune substances in the bodyfluids or tissue extracts of an animal which has acquired an active immunity against a tumor, as well as (6) the significance for immunity of cellular reactions in the host against tumor transplants and lastly (7) the presence in tumor cells of antigens other than organismal differentials.

(1) *Concomitant immunity.* From a theoretical point of view, this is perhaps the most important and most generally occurring type of active acquired immunity. It can be tested by inoculating animals, which already are the bearers of such transplanted tumors, a second time with tumor pieces and comparing the number of takes and the growth energy of the second tumors with those of the first. This immunity is elicited only if the organismal differentials of host and transplant differ, and is demonstrated the more readily, the greater the difference between the organismal differentials. It is very marked when a tumor grows for some time in a heterogenous host; homoiogenous tumors also give rise to immunity, but the growth of an autogenous tumor graft does not have this effect, nor is it observed if a spontaneous tumor is propagated through transplantation in the same closely inbred, homozygous strain in which it originated.

As we have seen in a preceding chapter, the experiments of Fleisher and the writer, as well as those of Haaland, prove that the growth of an autogenous spontaneous tumor does not influence the subsequent development of a homoiogenous inoculated tumor; conversely, the growth of a homoiogenous tumor does not affect the growth of autogenous tumor transplants and of metastases from the autogenous tumor.

However, in addition to differences in organismal differentials between host and transplant, other factors may enter into the production of concomitant immunity. This is clear if we compare the varying conditions under which this type of immunity has been observed. Stricker noted that at a certain period in the growth of a homoiogenous lymphosarcoma in a dog, immunity against a second inoculation developed, and Ehrlich found that the growth of a rapidly growing tumor inhibited the growth of a tumor of the same kind subsequently inoculated. According to Ehrlich and Schoene, the extirpation of the first tumor suspends this immunity and makes possible an inoculation with a second tumor. Ehrlich held that the first actively growing tumor used for its own growth all available growth-substances specifically required for the multiplication of tumor cells, and thus prevented the growth of a second tumor (athreptic immunity). That this interpretation does not

apply generally is indicated by the fact that the writer, as well as Jensen, observed that successive inoculation of tumors into the same animal led in some cases to the growth of both tumors; and Russell, as well as Tyzzer, found that there are actively growing tumor grafts which apparently do not produce immunity in the host. Russell distinguished, therefore, tumors which as a result of their growth, conferred concomitant immunity, and others which did not confer such an immunity. In addition, there were tumors which showed an intermediate behavior. However, there is reason for assuming that all growing homoiogenous tumors are able to induce active immunity against secondarily transplanted homoiogenous tumors, but that the degree of this immunity varies in different cases and that the presence of such an immunity may not always become manifest. If a tumor originates in a strain of animals in which, as the result of long-continued, very close inbreeding, the individuality differentials in the various animals have become very similar, then the individuality differentials of tumors which originate in such a strain are about the same as those of the animals constituting this strain, and such tumors after transplantation into members of this homozygous strain behave, therefore, about like autogenous tumors, which have no antigenic power.

After transplantation into a strange, closely inbred strain, the large majority of tumors do not show continued growth; they may grow for a short time and then retrogress and disappear, or may show a slightly longer initial growth. Similarly after transplantation into only partly inbred strains, not yet approaching homozygosity, the majority of primary (spontaneous) tumors do not take. But as we have discussed already in the preceding chapters, different tumors differ very much in this respect. There are some tumors which can be transplanted into almost all homoiogenous animals and others which may be transplanted in various proportions into such animals. Furthermore, there exist differences between different strains of animals serving as hosts; a certain tumor which originated in an American mouse may be transplanted into a considerable number of American mice, but not at all or only into a very small number of some European strains. These differences in transplantability depend partly on the relations and the degrees of mutual strangeness between the individuality differentials of hosts and transplants; but there enter also other factors, such as the growth momentum of the tumors, variations in their sensitiveness and power of resistance to injurious substances, and lastly, in their ability to adapt themselves to new hosts bearing individuality differentials of different degrees of strangeness. Moreover, different strains of hosts and different individual animals may not have the same ability to react against and to injure a transplant carrying a strange individuality differential. While the significance of these factors has not yet been analyzed sufficiently in the recorded series of transplantations, and while it is not yet possible to determine in most cases how much importance is to be attributed to one or the other of these factors, there is enough evidence at hand to warrant the conclusion that they play a role under various conditions.

To return now to the discussion of concomitant immunity. We have seen that the prerequisite for the development of this type of immunity is a differ-

ence in the individuality differentials of host and transplant, which makes it possible for constituents of the transplant to act as antigens in the host. Inasmuch as the constitution of the individuality differential depends upon the genetic constitution of the organism which is the bearer of the individuality differential, it may also be stated that certain genetic differences between host and transplant make it possible for constituents of the latter to act as antigens and to call forth processes of immunity in the host which become manifest if repeated transplantations of homoiogenous tumors are made. It should then be possible to demonstrate the presence of concomitant immunity in all cases in which a tumor grows in an animal whose individuality differential differs to a sufficient degree from its own. Experience, however, indicates that only in certain cases in which a homoiogenous tumor grows in an animal bearing a different individuality differential, can such a concomitant immunity be shown. In other cases, the presence of immune processes becomes apparent in an animal only after the successfully growing tumor has been completely extirpated, and in still others, only when the tumor growth comes to a standstill and, in the end, the tumor retrogresses. In the latter instance, after such a retrogression has taken place, immunity against a second homoiogenous transplant can be shown to exist. It does not appear probable that in these different types of immune reactions we have to deal with entirely different processes; it is much more likely that they are merely quantitative variations of the same fundamental process. It is possible that when a concomitant immunity becomes manifest, the amount of immune substances produced as a result of the action of the strange antigens is sufficiently great to make possible the demonstration of these immune processes, notwithstanding the presence of a growing tumor, which seems to have the tendency to absorb a certain quantity of immune substances and to make them innocuous. We may also assume that another type of tumor may absorb so great a proportion of the immune substances that the immune bodies remaining free in the circulation of the host are unable to prevent the growth of a second homoiogenous tumor transplanted at a time when the first one is already growing. Extirpation of the first growing tumor would then make immune substances available for the attack on the second tumor. In some instances, in which the individuality differentials of host and transplant possess a sufficient degree of strangeness, the amount of immune substance produced in response to the first homoiogenous growing tumor may become so large that it gradually begins to inhibit and prevent the further development of the first graft, which then ceases to grow and may even retrogress. The absorption of this tumor material would still further increase the strength of the immune processes, so that after retrogression of this tumor the animal has become completely immune against a further transplant of a homoiogenous tumor. But there are other cases in which, after extirpation of a tum[illegible]
becomes so weak th[illegible]
transplantation of a [illegible]
interpret the various types of immunity which can be distinguished as manifestations of the same basic process; the differences noted would then be due

merely to quantitative differences in the intensity of the immune reactions in various animals or strains, in the ability of different kinds of tumors to neutralize the immune substances, and in the power of resistance of different tumors to the injurious action of such substances. The immunity found after extirpation of a growing tumor or after retrogression of a formerly growing tumor would then represent merely quantitative variants of the same type of immunity.

There exist certain other experimental procedures which may make it possible to prove the existence of immunity against a tumor graft. This may in some cases be accomplished by experimentally weakening the second tumor in vitro, previous to inoculation. Also, by artificially weakening a first tumor it may be possible to demonstrate the development of an active immunity, because under these conditions the absorbing and neutralizing function of the tumor may be markedly diminished. Through experimental weakening of the second tumor by means of graded application of heat previous to transplantation, Fleisher, Corson-White and the writer demonstrated the inhibiting effect of the first tumor on the development of a subsequently transplanted tumor, in mouse carcinoma No. IX, in which, in successive transplantations of fully active, unheated tumor pieces, immune processes are not manifest. Thus it could be shown that a first unheated tumor possessing its full growth energy prevents the growth of a second tumor which has been exposed to a temperature of 44° for a period of from thirty-five to forty minutes. It does not entirely suppress, but it weakens the growth of a second tumor which has been exposed to a temperature of 44° for thirty minutes. If the growth energy of the first tumor has also been slightly reduced through heating, the development of a second tumor is prevented only if its growth energy has been diminished quite markedly through heating for forty minutes. But if the first tumor had been injured through heating as much as the second tumor, or even more, we then observed in several instances the opposite phenomenon, namely, an increase in the growth energy of the second tumor. Thus one tumor may, under certain conditions, have a beneficial influence on the growth of a second tumor, perhaps owing to a neutralizing effect on substances antagonistic to tumor growth which a first, weakly-growing tumor may exert.

It seems that the antigenic function of a tumor graft bears some relation to the intensity of its metabolic activity, or to the presence of substances which are readily injured by heat even of a moderate intensity. The influence which the second tumor exerts on the first is less marked, but an enhancing effect of a second, less inhibited tumor on a first, weakened tumor has been observed also by Andervont in the case of sarcoma 180. Under other circumstances, however, a second tumor whose growth energy has been only moderately diminished through heating may be victorious in competition with a first, more markedly depressed tumor; and it is further possible to produce experimentally a balancing between a first and a second tumor. Apparently the interaction of two mutually antagonistic processes may play a role in bringing about this effect, namely, (1) the production of immune substances in the host, and (2) their absorption and neutralization by the tumor, or perhaps by organismal

differentials which have been given off by the tumor into the circulation and which act as antigens. Also, Seelig and Fleisher observed such a balancing between the growth energy of the first and second tumors and they noted that the tumor with the greater growth energy has the advantage over a weaker tumor. In addition, they found that intraperitoneal inoculation with tumor material may exert a greater immunizing power than a subcutaneous inoculation, although the intraperitoneal inoculation may not be followed by actual growth of the carcinomatous tissue. In this case we may have to deal with an immunity similar to that which is caused by inoculation of normal or tumor tissues which do not noticeably grow.

This method of using an originally active, virulent tumor, after its growth energy has been experimentally reduced, for the demonstration of immune processes which otherwise would not be manifest, was subsequently employed also by Tsurumi, as well as by Rohdenburg and Bullock, and in a modified way by Caspari and his collaborators, Schwarz and Ascoli. Besides grading the growth energy by means of heat, they accomplished the same purpose also by exposing the tumors to the action of radiation or of various chemicals. Only when the inoculated tumor material was living did they find an immunizing effect. Presumably the injured tumor grew temporarily to a slight extent, but it soon retrogressed, and it is possible that the immunization was accomplished by living but not growing material. However, the essential point is that a balancing may take place between the first and second tumor pieces in accordance with the degree of potential growth energy which each inoculated piece possesses; and Caspari and Ascoli also observed such an effect. The greater the growth energy of the first piece, the more it tends to diminish the growth energy of the tumor developing from the second piece; furthermore, the influence of the first piece is inversely proportional to the growth energy of the second. In a similar manner Lumsden has recently demonstrated that by weakening the growth of a second transplanted piece of cancerous tissue in various other ways, such as by inoculation in unfavorable places, constriction of the blood vessels leading to the tumor, by means of ligatures, and injection of formalin into the transplant, it is possible to prove that tumors like the Twort mouse carcinoma or mouse carcinoma 63, which, according to Russell, belong to the type of tumors which do not elicit concomitant immunity, may give rise to such an immunity. Of interest is the observation of Foulds that parallel to changes in their growth energy, tumors in the course of continued transplantations may undergo variations in their power to elicit concomitant immunity. The effects which a first growing tumor exerts on a second one may further depend also on the kind of tumors used. For instance, the growth of a secondarily transplanted mouse chondroma, a very slow-growing tumor, was apparently not affected by a first tumor of the same kind, because these cartilage tumors are very resistant to injurious influences.

We may therefore conclude that even tumors whose growth apparently does not lead to the development of immune processes, may actually have this effect provided their individuality differential differs from that of the host. The existence of concomitant immunity may then, in general, be ascribed to

differences between the *individuality differentials* of the host and transplant. On the other hand, Caspari and Schwarz believe that concomitant immunity is due to necro-hormones given off by the growing tumor and that the greater immunizing power of a rapidly growing tumor is dependent on the more extensive necrosis which occurs in the central portions of such tumors.

(2) *Immunity following extirpation of a tumor.* We have already referred to this kind of immunity as representing a variety of concomitant immunity, which becomes manifest only after extirpation of the first tumor. Uhlenhuth, Haendal and Steffenhagen have shown that when homoiogenous rat sarcomata growing in rats were excised, the rats were thereby rendered immune to re-inoculation with this type of tumor. But, if the operation was incomplete and the tumor recurred, a second inoculation was successful. There has been much discussion concerning this experiment; some have denied its significance, or even its occurrence. However, Fleisher and the writer were able to confirm the findings of Uhlenhuth and his collaborators; after extirpation of a homoiogenous mouse carcinoma No. IX, the animal became immune against reinoculation with this tumor. It is to be noted, however, that the growth of this type of carcinoma did not, under normal conditions, lead to the manifestation of a distinct concomitant immunity, presumably because the immune substances are absorbed by the growing tumor. In addition, it must be assumed that the production of immune bodies continues for some time after the source of the antigens has been removed. The absorptive or neutralizing function of a first tumor is not exercised by an autogenous, so-called spontaneous tumor, the extirpation of which does not elicit processes of immunity either against re-inoculation with autogenous or with homoiogenous tumors. We may therefore conclude that the organismal differentials are involved also in these neutralizing mechanisms and that, in particular, homoiogenous individuality differentials are able to neutralize homoiogenous immune substances.

(3) *Immunity following retrogression of tumors.* Homoiogenous tumors may grow for some time and then retrogress apparently spontaneously; if the tumor pieces used for inoculation have been subjected to chemical or physical injuries previous to transplantation, such a retrogression is particularly apt to occur. In all these cases retrogression takes place because conditions injurious to the tumor cells have had a depressive effect on the tumor growth. We described a spontaneous retrogression of transplanted tumors in 1901. We observed also that during the first stages of retrogression mitotic cell proliferation may still proceed quite actively in the tumor cells and that tumors in the early stages of retrogression may be transplanted successfully and may subseqently recover their full vigor of growth; but at later stages of retrogression mitotic proliferation is much reduced or it ceases altogether, and from then on the ability of the tumor to recover after renewed transplantation is very much decreased. These observations were subsequently confirmed and extended by Woglom, and in 1905 Clowes and Baeslack established the interesting fact that mice, in which a homoiogenous mouse carcinoma had retrogressed, had become immune against re-inoculation with a homoiogenous tumor. In the case of heterotransplantation, for instance, if a mouse tumor

is grafted into a rat, the tumor, as a rule, after a temporary growth retrogresses, and then a second inoculation of a similar tumor into the same host does not lead even to the limited growth shown by the first transplant. The animal has become immune as the result of the growth and retrogression of the first tumor. In accordance with what we know as to the inability of autogenous tumors to elicit immunity of this kind, is the great infrequency with which spontaneous tumors retrogress; but if they do retrogress, this is presumably brought about by factors other than immunity, or by an immunity not directed against the organismal differentials but against other substances.

As stated above, we must conceive of the immunity following retrogression of a tumor as a variety of concomitant immunity, which primarily is due to the dissimilarity and incompatibility of the organismal differentials of tumor and host. As the result of this incompatibility the primary, preformed homoio- or heterotoxins of the host injure the transplant and the strange individuality or species differentials of the grafted tumor may act as antigen, eliciting the production of immune bodies, which then support and complete the effect of the primary homoio- or heterotoxins; and it is probable that these immune substances are mainly responsible for the injury of the transplant and the subsequent cessation of its growth and its retrogression. In addition, during retrogression of the tumor, tumor material is being absorbed, which also may serve as antigen and cause additional formation of antibodies.

The retrogression immunity is very effective and may cause the shrinking and the ultimate disappearance of tumors which had already been established in the host, and which had successfully passed through the early, dangerous stages following transplantation. As a result of this immunity the tumor itself, which has given rise to the production of the immunity, experiences the effect of its own activity and undergoes complete retrogression. A subsequent second inoculation of a tumor piece is then unsuccessful. After the tumor has once been absorbed, the animal organism is no longer able to neutralize the immune substances which may still continue to be produced. We believe, therefore, that also this type of immunity, against the growth of a transplanted tumor, is not merely caused by the retrogression of a tumor, but that it already sets in some time preceding the retrogression and continues during this process, and that it is intensified through the absorption of material from the retrogressing tumor. There follows then a struggle between the host, which produces substances injurious to the tumor, and the inherent growth energy of the tumor combined with its power to neutralize injurious substances; in addition, there may perhaps come into play also certain adaptive processes in the cancer cells.

The increase in immunity which takes place during the retrogression of the tumor must in some way depend on the activity of the living, metabolically still potent tumor cells. This is indicated by the fact that when we produced retrogression of a first mouse carcinoma IX by exposing the tumor, previous to transplantation, to a degree of heat sufficient to injure the tumor cells markedly and thus experimentally to induce the subsequent retrogression of the grafted tumor, the immunity resulting from this retrogression was

less pronounced than that following a spontaneous retrogression of a larger tumor which at first grew well. Lumsden similarly observed that the immunity is greater after retrogression of large than of small tumors. It may be assumed that in order to accomplish the retrogression of an actively growing large tumor, a higher degree of immunity will be required than for the retrogression of a weak tumor, and more immune substances will therefore subsequently be available for combating the growth of a second tumor. As stated, this high degree of immunity necessary for the absorption of large tumors is achieved only if the individuality differentials of host and transplant are sufficiently different to cause a primary incompatibility, which must, however, not be so great that it prevents growth of a tumor during the early periods following transplantation. Subsequently, it must be assumed, the quantity of immune substances increases at a rate too rapid for the neutralizing power of the tumor. However, we have seen that also the immunity induced by a growing tumor is greater if the first tumor grows vigorously, than if it has been weakened in its growth intensity.

That immune substances are produced under these circumstances and may be present in the spleen of the tumor-bearing animals is indicated by the experiments of Mottram and Russ, as well as of Woglom, who show that in the spleen of animals in which tumors had retrogressed, substances are present which injure the cells of a similar tumor. As mentioned previously, Woglom's recent investigations suggest the possibility that the antibodies circulating in the bodyfluids of an immune animal can be absorbed by tumor mash. These substances, then, are able to injure in vitro a piece of tumor and prevent its successful transplantation; and likewise Lumsden has found that substances circulating in the blood of rats, rendered immune against rat sarcoma, succeeded in killing cancer cells as well as spleen cells of the same species growing in vitro. As stated in a preceding chapter, Lumsden attributes these effects to "antimalignancy" immune bodies, devoted as a result of the growth of a homoiogenous sarcoma; but Phelps and others interpret them as due to cytotoxins, which form in response to the presence of strange species or individuality differentials, since these reactions occur also with normal spleen cells and may be elicited by antigens present in normal spleen.

In addition to cytotoxins, there may be found in the bearers of retrogressed tumors hemagglutinins, which may, however, affect not only erythrocytes, but also other kinds of cells (Gorer, Lumsden). Lumsden has made it probable that these cytotoxins and hemagglutinins are distinct and develop independently of each other; he also observed a definite relation between the strength of such antibodies and the retrogressive changes which take place in the tumor; this would indicate that retrogression is due to the action of immune substances. However, while it is quite probable that the latter aid in the injury of the tumor, it is probable also that primary mechanisms are involved, and that the immune substances originate as a reaction against primary incompatibilities between organismal differentials.

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which is present also in rat sarcoma cells, as demonstrated by the agglutination of these rat red corpuscles when mixed with the serum of a rat made immune against a rat sarcoma. If pieces of rat sarcoma are transplanted into such a rat, immune substances would not be produced, because of the presence of a common antigen in the sarcoma cells and in the host cells, and hence an inoculated piece of rat sarcoma would grow. In this case an agglutinogen present would, therefore, make possible the growth of a transplanted tumor and would actually function as a susceptibility factor for this tumor. We have discussed these observations already in a preceding chapter.

The histological changes which are observed around retrogressing tumors do not explain the character of the immunity noted in these animals. Thus Gaylord and Clowes, and others, found necrosis and hemorrhages, as well as collections of lymphocytes in or around such retrogressing tumors; the presence of lymphocytes may be taken to indicate an active reaction on the part of the host against the transplant. However, Ishii and the writer, in examining tumors that retrogressed following an in vitro exposure to heat, did not observe such collections of lymphocytes; there was merely a connective tissue capsule around the tumor and a replacement of parts of the tumor by fibrous tissue.

It is an interesting problem as to whether the immunity thus produced is specific for the tumor which has retrogressed, or whether it applies also to other types of tumors. Bashford assumed it to be specific, because he noticed that mice in which an immunity had developed after retrogression of a carcinoma were immune against a re-inoculated carcinoma, but not against a sarcoma. However, as Caspari and others have pointed out, this result is probably to be explained by the difference between the growth energy of the mouse sarcoma and that of the carcinoma; an immunity sufficient to prevent the growth of a carcinoma, may not have been sufficient to prevent that of a much more active sarcoma. In general, the specific immunity which has been acquired is directed against strange individuality differentials in case of retrogression of a homoiogenous tumor, and against a particular species differential after retrogression of a heterogenous tumor. The "retrogression" immunity is therefore essentially an immunity directed against organismal differentials which are common to various kinds of tissues and tumors belonging to the same species; it represents what Ehrlich called "pan-immunity," by which he meant an immunity directed not only against a tumor composed of a certain kind of tissue, but also against a tumor composed of another kind of tissue of the same species. Nevertheless, the immunity may be greater to tumors composed of the same kind of tissues than to another kind of tumor belonging to the same species; such a partial specificity was observed by Greene after a primary transplantation of homoiogenous tumors of the rabbit into the anterior chamber of the eye, and a second transplantation of the same or of another kind of tumor into the other eye or into the testicle. Under these conditions a growing first tumor seems to act similarly to a retrogressed tumor. In addition to the "pan-immunity" directed against all the individuality differentials of a species, there may exist, therefore, an immunity directed

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against the individuality differential of a particular tumor. This is indicated also by the observations of Rous and Murphy that the immunity noted after retrogression of three types of chicken sarcoma, namely, a spindle-cell sarcoma, an osteochondroma, and a rifted sarcoma, was directed mainly against the special kind of tumor that had retrogressed. It appears moreover that different types of tumors differ quantitatively in the degree of immunity they produce, and the effectiveness of the immunization seems also to depend on the place where the first and second tumors were inoculated. Thus, in the rabbit each tumor of the uterus and mammary gland seemed to differ in certain respects from the Brown-Pearce rabbit tumor, in experiments reported by Appel, Saphir and their collaborators, and by Cheever and Morgan, and by Greene. Furthermore, there is even the possibility that immunity against a tumor of a different species is not absolutely specific, but that it may extend also, although to a lesser extent, to individuals belonging to a nearly related species.

(4) *Immunity produced through inoculation af pieces of normal tissue or af tumor tissue unable ta induce tumor formation.*

Ehrlich obtained immunization against a mouse carcinoma by first inoculating mice with pieces of a hemorrhagic mouse carcinoma, which itself did not give rise to tumor formation because the tumor cells were injured. This experiment suggested the use of normal tissues for purposes of immunization. Bashford, by injection of homoiogenous defibrinated blood, obtained active immunity against subsequent inoculations with homoiogenous tumor, and Schoene found that other living tissues, in particular, embryonal tissues, were similarly effective. These observations were subsequently confirmed and extended by many investigators. The results obtained may be summarized as follows: (a) Only living tissue is effective in inducing immunity; dead cells do not immunize. (b) It is the organismal differentials of the inoculated pieces which give rise to this type of immunity. There must be a definite relation between the organismal differential of the piece of tissue serving as antigen (vaccine) and that of the host; furthermore, the organismal differential of the antigen must bear a definite relation to the differentials of the tumors against which the immunization is directed. Autogenous tissues do not, therefore, act as an efficient antigen (Apolant, Woglom). Tissues which possess homoiogenous differentials in reference to the host animals, immunize against homoiogenous tumors, and heterogenous tissues immunize against tumors possessing the same heterogenous differential. However, it is possible that an immunization may in certain instances be produced also by the use of tissues from nearly related species, mouse tissue immunizing against a rat tumor and vice versa; but this immunity, if successful at all, is much weaker than that produced by tissues with identical organismal differentials. (c) Different types of tissues differ as to their effectiveness as antigens; embryo skin is very effective, whereas cartilage, bone, muscle tissue, lens and brain are not. Other tissues range between these extremes. That cartilage, bone and muscle are relatively unfavorable may be due to the predominance, in these tissues, of inter-

cellular substances, and we have every reason to believe that it is the cytoplasmic elements which produce most actively the individuality differentials rather than the paraplastic parts, although there is some evidence that also the latter are not devoid of individuality differentials. Correspondingly, we have found that normal cartilage elicits a weaker antagonistic reaction of the host than does thyroid, which is rich in cells. As to the inefficiency of lens and brain, this might be expected, because they do not furnish very effective homoiogenous antigens in immunization experiments, although as we have seen in an earlier chapter, they do contain homoiodifferentials. (d) There is, then, ample reason for the conclusion that it is the organismal differentials, and in particular, the individuality differentials, of the normal tissues acting as antigens, which call forth immunity against the organismal differentials of the tumor.

However, it is not necessarily the specific individuality differential of a certain individual which serves as antigen against the identical individuality differential in the tumor; but any individuality differential of the normal tissue, which differs from the differential of the host animal and is therefore strange to the latter, may function as antigen for the tumor. It may then be assumed that any strange individuality differential activates, in the host, reactions which are directed against all other strange individuality differentials, provided both host and donor belong to the same species.

We have seen that autogenous tissues cannot serve as antigens against a subsequently transplanted homoiogenous tumor; likewise, it may be safely assumed that tissues fom an animal belonging to a closely inbred strain cannot function as an effective antigen in another animal of the same inbred strain. The experiments of Eisen and Woglom on transplantation of a mammary gland carcinoma in inbred strains of rats, to which we have already referred in a preceding chapter, as well as certain experiments in which the production of immunity was attempted against transplanted leukemic cells, are in agreement with this conclusion. Rhoads and Miller observed that implantation of normal mouse tissues may immunize mice against inoculation of mouse leukemia; but while, according to MacDowell, embryo-skin of the inbred strain Sto Li can produce resistance in strain C58 to leukemic cells of line I, embryo-skin of strain C58 is not able to serve as an efficient antigen. However, embryo-skin from hybrid (C58 x Sto Li)F_1 may call forth immunity in strain C58. Evidently a single gene set of Sto Li present in the hybrid enables the tissue of the latter to function as antigen and the presence of the gene set of C58 in the hybrid does not interfere with the antigenic action of the strange Sto Li gene set. In a similar manner, it seems that the agglutinogens of the F_1 hybrid between two closely inbred strains, which has inherited a gene set from each of the parents, possess the ability to produce antibodies (agglutinins) in certain hosts and to absorb these agglutinins; in this case, also, one strange gene set is sufficient for this purpose and the double gene set is not required. (e) While a strange individuality differential of normal tissue may call forth immunity against a transplanted tumor, the immunity thus produced is not so strong as that following retrogression of a tumor. It

is even less than the usual concomitant immunity and the degree of resistance following extirpation of mouse carcinoma IX. (f) Considering the relative weakness of the immunity obtained by inoculation of normal tissue, it might be foreseen that this type of immunization is ineffective against a tumor possessing a great growth energy, or one as resistant to injurious conditions as the chondroma of Ehrlich. Rous and Murphy were not able, therefore, to produce immunity by inoculation of normal tissues against the Rous chicken sarcoma, although during retrogression of these tumors there developed spontaneously an immunity which seemed to be directly against the agent.

(5) *The demonstration of immune substances in the bodyfluids or tissue extracts of animals which have been inoculated with cancerous material or in which cancer has grown.*

In the blood of an animal which has become immune against certain bacteria, either by recovering from the disease caused by them or by vaccination with the microorganisms or certain parts of them, the presence or antibodies directed against these bacteria can in many cases be demonstrated. Similarly, antibodies have been produced against proteins and against cells belonging to a different species, and in some instances even against certain cells belonging to the same species but to different individuals, or against certain kinds of tissues and organs characteristic of a species. It is natural that following the proof that an active immunity can be produced against transplanted tumors, the question should have been raised as to whether there are indications in this case, also, of the presence of antibodies circulating in the blood or retained in the tissues. The existence of such antibodies against heterogenous tumors can be shown, but it has been impossible, at least until more recently, to demonstrate antibodies which developed against homoiogenous tumors.

In the large majority of cases, attempts to reveal the presence of antibodies in the blood of immune animals directly, by injecting such blood into susceptible animals of the same species and thereby inhibiting the growth of a tumor graft, did not succeed, although a few investigators (Clowes, Beebe and Gaylord, and C. Lewin) have reported positive results. In subsequent experiments, Lumsden believed he had obtained some positive results by injecting serum of heterogenous animals, immunized against a tumor, into the tumor itself growing in homoiogenous animals, but under conditions which were not favorable to a rapid proliferation of the cancerous tissue.

By the use of in vitro cultures of tumor cells, Lambert and Hanes found antibodies against cancer cells in the serum of heterogenous, but not of homoiogenous, animals immunized against this tumor. Likewise, Yamagiwa believed he had demonstrated antibodies against mouse tumors in the extract of spleen of rabbits immunized against such tumors. Also, in experiments of Tyzzer there was some indication of an immune substance in the serum of hybrid F_2, F_3, and F_4 mice, between Japanese susceptible and white mice non-susceptible to a tumor which had originated in a Japanese mouse. The hybrids were non-susceptible to this tumor. If a piece of this tumor was

inoculated into a susceptible Japanese mouse and serum from the hybrid injected into the same mouse, but not directly into the tumor tissue, necrosis increased in the graft, the mitoses were diminished, and polymorphonuclear leucocytes infiltrated the transplant. These changes indicated an injurious effect of the serum, which was, however, only transitory; subsequently the transplanted piece began to grow.

However, in animals belonging to the same species as the tumor transplant it has not been possible to demonstrate the existence of such antibodies until recently. Older experiments of Lambert and Hanes had been negative; also the work of Peyton Rous with parabiotic rats—a susceptible rat joined to a rat naturally immune to a rat tumor—failed to reveal immune bodies in the susceptible animals, while other investigators were unable to find that homoiogenous blood serum of actively immune animals affected the growth of tumors, even if the serum was injected previous to or soon after the implantation of the tumor pieces. Contrary to these results are those of Lumsden, who noted indications of the presence of antibodies against rat sarcoma in rats actively immunized against this sarcoma, or in which the tumor had retrogressed. We have referred already to these experiments in which the serum was added to tissue cultures of rat sarcoma and rat spleen, and we have likewise discussed the experiments of Woglom, which strongly suggested that in serum of a rat, in which a tumor had retrogressed, substances are found which injure the tumor cells and may prevent their growth after inoculation into a homoiogenous animal.

Furthermore, substances injurious to Rous chicken sarcoma were obtained, not only in geese, ducks, rabbits and goats actively immunized against this tumor, but also in fowls bearing a slowly growing fibrosarcoma, immune substances developed very gradually which neutralized in vitro not only the agent of the fibrosarcoma but also the agent of the more rapidly proliferating Rous chicken sarcoma. However, these latter manifestations of tumor immunity are different from those observed in the case of mammalian tumors, because this immunity in avian tumors was primarily directed not against the tumor cells as such, but against the agent which causes the sarcoma to grow.

If we compare the reactions of a host against normal tissues and against tumors, both possessing organismal differentials differing from those of the host, the bodyfluids are found to contain substances which are injurious to both kinds of grafts. There are strong indications that preformed substances as well as newly formed, immune substances, directed against the individuality or species differentials of these transplants, are active, and that the substances directed against the strange species differentials are stronger than those directed against strange individuality differentials. But whereas in the case of normal tissues the immune substances are apparently of minor importance, in the case of tumors they appear to play the major role in determining the fate of the transplants; still, even in the latter it is primarily the divergence between the organismal differentials of host and transplant which makes it possible for the differentials of the tumor grafts to function as antigens.

In principle, there do not seem to be significant differences in the antigenic

function of transplanted tumor cells and normal cells and of injected erythrocytes, which latter elicit the formation of hemagglutinins and hemolysins. The differences which do exist seem to be mainly of a quantitative nature, the immune substances, which are formed after transplantation of living normal tissues, being weaker. There is, perhaps, the additional difference that the growing tumor seems to be able to absorb and to neutralize very effectively the immune substances circulating in the host, while normal tissues and erythrocytes do not do so. The proportion of neutralized and non-neutralized immune substances and the power of resistance of the tumor tissue to the absorbed immune substances seem to vary in the case of different tumors, and it is largely these variations which are probably responsible for the differences in the types of immunity which develop after transplantation of tumors into hosts whose organismal differentials differ from those of the tumor. However, as we have stated already, there are indications that not only the organismal differentials, but also other constituents of the tumor cells, may give rise to states of immunity and to the production of immune substances; some of these data we shall discuss in the last part of this chapter.

The presence of immune substances in the circulating bodyfluids of an animal actively immunized against a transplanted tumor is perhaps suggested also by the demonstration of the presence of substances in the circulating bodyfluids which enable a tumor graft to grow in an animal belonging to a strain unfavorable to the growth of a particular type of tumor. This has been demonstrated by means of parabiosis, if an individual belonging to a strain favorable to the growth of the transplanted tumor was joined to an individual normally resistant to the growth of the inoculated tumor. Substances supplied by the first partner enabled the tumor to grow in the second otherwise unsuitable partner. We have referred already to experiments of this kind by Zakrzewski and of Cloudman in a preceding chapter, when we discussed factors in the growth of transplanted tumors. However in this case we have to deal with substances favoring tumor growth while here we are concerned with substances inhibiting tumor growth.

(6) *Cellular reactions of the host against the tumor transplants.* We have discussed evidence which tends to prove that under various conditions immune substances directed against the individuality or species differential of the transplanted tumor may develop in the host. But, in addition, certain types of host cells react against the transplant, and these reactions manifest themselves locally around the graft as well as in the circulation of the host. The local reactions consist, above all, in the accumulation of lymphocytes, but also polymorphonuclear leucocytes, connective tissue and blood vessels may take part in these processes.

Simultaneously with the early studies of the role of lymphocytes in transplantation of normal tissues there began the study of the role of various types of leucocytes, including lymphocytes, in the reactions of the host against a tumor. But the cellular changes against transplanted tumors were interpreted as the local manifestations of a general immunity against the tumor growth. This immunity was considered as distinct from other types of immunity,

although it was conceded by some authors that also immune reactions, which were analogous to those developing against embryonal tissues, may participate in this process. Other investigators believed that the acquired resistance or immunity against tumor transplants led to a deficiency in the ingrowth of stroma from the host into the tumor. Under normal conditions the surrounding host tissue supplies the tumor with blood vessels and a connective tissue stroma; but it was assumed that if the host has been made resistant or immune against the transplant, it fails to provide this stroma.

As to the lymphocytes, in the case of normal tissue transplants we found a double significance of these cells: (1) Under certain conditions the strength of the lymphocytic accumulation could be used as a quantitative measure of the intensity of the reaction of the host against a strange individuality differential; it served therefore as a standard with which to measure the difference between the individuality differentials of host and donor, and, accordingly, also of their degree of relationship or strangeness. (2) The lymphocytes, in collecting around the transplant and invading it, were able to injure it if they penetrated into it in dense masses. On the other hand, we did not find any evidence for the further conclusion that the lymphocytes give off substances which diffuse into the transplant and thereby damage it, an assumption that was made by some investigators in the case of tumor transplants.

The role which lymphocytes play in the growth and retrogression of transplanted tumors seems to be similar to that seen in the case of transplanted normal tissues. There is, however, one significant difference. While the marked accumulation of lymphocytes around normal tissues and their invasion of these tissues may lead to the injury and destruction of a considerable part of the graft, in the case of a growing tumor the multiplication and expansive growth of the tumor cells may be so active that the lymphocytes cannot overcome the graft. Also, around retrogressing tumors the local accumulation of lymphocytes does not need to be very conspicuous. This condition accounts perhaps for the fact that in the transplantation of tumors several investigators did not attribute to the lymphocytes the role which we did in the grafting of normal tissues, but they considered them, rather, as important agents in the production of the general immunity which develops under various circumstances against tumor transplants. This latter interpretation seemed also to be supported by the observation that while an accumulation of lymphocytes may become noticeable already after a first inoculation of a piece of tumor, it is more accentuated and it appears more rapidly after a second inoculation, because here the inoculation takes place in an animal in which immunization processes have set in already as the result of the first inoculation.

As stated, the activity of lymphocytes around a piece of tumor does not need to be pronounced; this is true especially if the first transplanted piece begins to grow actively soon after transplantation. An accumulation of lymphocytes was more marked in the early experiments of Burgess and Tyzzer; however, these investigators did not study the local reaction around homoiogenous tumors, but around pieces of tumor which approached a heterogenous character; and here polymorphonuclear leucocytes were as prominent as

lymphocytes, an observation which agrees with our findings that polymorphonuclear leucocytes tend to collect around heterogenous transplants of normal tissues. Burgess and Tyzzer noted that if the tumor was not destroyed too rapidly through the accumulation of these wandering cells, dense scar-like fibrous tissue was produced in the transplant. Likewise, in a later analysis of these phenomena, Tyzzer (1916) did not interpret these primary cellular reactions as due to and directed against incompatible organismal differentials, which secondarily call forth an immunization, but he adopted Russell's view that natural resistance against tumors is merely the ability of the host to acquire an active immunity against the tumor, while susceptibility means the lack of this ability. Tyzzer assumed, therefore, that in every case the local cellular reaction was due to an active immunity produced against the transplant, and it was considered as a phenomenon specific for tumors.

As to the mechanism underlying this reaction, Tyzzer assumed that in combination with an immune body the tumor products become strongly chemotatic for leucocytes and at the same time stimulate the surrounding fibroblastic tissue. In animals already immunized, the reaction not only sets in more promptly, but here also the polymorphonuclear leucocytes are more numerous, while in as yet untreated animals, in which the immunity develops only gradually following the first inoculation, the reaction takes place more slowly and the lymphocytes are found in relatively larger numbers; the preponderance of lymphocytes signifies a milder reaction on the part of the host. In addition to the movements of lymphocytes and polymorphonuclear leucocytes, an increase in the number of fibroblasts occurs in the surrounding tissue, and this he compared with the formation of granulation tissue in inflammatory processes. Tyzzer concluded, then, that immunization leads to the production of sensitizing antibodies in the host, and these combine with a substance given off by the tumor to form an injurious substance (anaphylatoxin), which injures the host tissue surrounding the tumor. As a result of such injury, inflammation sets in and lymphocytes, polymorphonuclear leucocytes or monocytes appear. In general, it is the presence of this antigen-antibody combination (anaphylatoxin) which causes the accumulation of the leucocytes of the host in and around the graft.

Also, in the case of other tumors cellular reactions around the transplants were noted and the resistance of the host to transplanted homoiogenous tumors was attributed especially to the lymphocytes. Thus, when chicken sarcoma was transplanted into a naturally immune fowl, Rous and Murphy observed on the fifth day following transplantation the appearance of masses of lymphocytes around the graft, which then degenerated. In other instances it seemed, however, that the tumor was already seriously injured at an earlier period following transplantation, owing to the failure of the surrounding tissue to provide a stroma for the graft. Similarly, Mottram and Russ, studying immunity against Jensen rat sarcoma, found that when a piece of tumor was inoculated into non-immunized rats, the lymphocytic reaction which developed around the transplant was very slight and did not seriously interfere with the growth of the tumor. But if, following a first inoculation with experimentally

weakened tumor pieces, a second non-weakened piece was inoculated, there occurred on the second and on the third day a marked accumulation of lymphocytes, the sarcoma cells disappeared rapidly, fibrous tissue formed subsequently, and also plasma cells were seen. With the disappearance of the sarcomatous tissue the lymphocytic reaction came to a standstill.

However, preceding these latter investigations Da Fano, in 1910, had emphasized the significance of lymphocytes and plasma cells in tumor immunity, but his interpretation of the function of the lymphocytes differed from that of Tyzzer and also from our conception. Da Fano noted an accumulation of lymphocytes not only around transplanted pieces of tumor, but these cells as well as plasma cells were seen also in various other places as for instance in the connective tissue underneath the skin of the animal during the process of immunization. Only living tumor tissue elicited this reaction; furthermore, it was lacking around a second piece of tumor inoculated in an animal in which immunity had already developed. Da Fano attributed, therefore, to the lymphocytes and plasma cells the function of initiating the general state of immunity which follows the inoculation of a piece of homoiogenous normal tissue or tumor. Somewhat later, Baeslack observed in addition to the localized reaction, a general reaction of the lymphocytes to tumor growth; the active growth of a homoiogenous tumor was accompanied by a decrease in the number of lymphocytes and by an increase in the number of polymorphonuclear leucocytes, whereas the retrogression of a tumor was associated with an increase in the number of lymphocytes as an indication of the development of immunity against the transplanted tumor. Quite recently, Lewis has confirmed the increase in the number of polymorphonuclear leucocytes in the peripheral circulation in mice in which the transplanted tumor grows.

The most extensive and ingeniously varied investigations concerning the relations between tumor immunity and activity of lymphocytes were carried out by Murphy and his associates. They noted both a local accumulation of lymphocytes around the tumor graft as well as a general increase of lymphocytes in the circulation following transplantation; an injurious effect resulted, however, from an increase in the number and activity of lymphocytes, not only in homoiogenous and heterogenous, but also in autogenous tumor grafts. After heterotransplantation of a piece of tumor, the lymphocytic reaction played the principal role in the destruction of the transplant. These investigators concluded, furthermore, that immunity against a tumor can be elicited not only through inoculation with a piece of tissue or of tumor belonging to the same species, but that any non-specific agency which increases the number and activity of lymphocytes, increases thereby the defensive reactions of the host against the transplant, and conversely, any agency that decreases the number and activity of lymphocytes increases thereby the susceptibility of the host to the tumor transplants. Small doses of Roentgen rays stimulate and strong doses injure the lymphocytes; exposure of mice to graded intensities of dry heat and injections of certain oils or unsaturated fatty acids stimulate the lymphocytes. The effects of these various agencies on

resistance and susceptibility to tumor growth can be gauged by their effects on the lymphocytes. In accordance with the views expressed by Da Fano, Murphy believes that the lymphocytic reaction which develops around a tumor graft in an immunized animal is the local manifestation of a general reaction which takes place in the animal. When mice are naturally immune against a tumor graft, or when they have been made immune by experimental means, they show an immediate and very marked increase in the number of circulating lymphocytes following inoculation with a piece of tumor against which they are immune, whereas, the other blood cells show no change. Similarly, in the lymph glands of a mouse which has been immunized experimentally, or in which immune processes set in following absorption of its tumor, the mitotic proliferation of the lymphocytes is much increased, and it is still further increased after a second inoculation of a tumor piece. From all these observations Murphy concluded that the general, as well as the localized lymphocytic reaction is not merely a condition accompanying tumor immunity, but that it is responsible for the development of this immunity, and as stated above, he found that even an otherwise successful autogenous transplantation of spontaneous tumors can be prevented through an increase in the activity of lymphocytes.

There were various other experiments which seemed to support this interpretation. Thus Murphy found that if pieces of heterogenous (mammalian) tumors or embryonal tissues were transplanted on the chick allantoic membrane, they grew for some time. However, if he transplanted simultaneously with the mammalian tumor small pieces of chicken spleen or bone marrow, the tumor did not grow, presumably because of the injurious action of the lymphocytes contained in the latter organs. Accordingly, growth of the tumor ceased at the time when, in the eighteen or twenty-day-old chick embryo, the spleen begins normally to function. But, Danchakoff maintains that it is not the lymphocytes of the transplanted spleen or bone marrow which grow out towards the tumor and injure it, but monocytes or reticulo-endothelial cells.

There are other regions in the body where the resistance offered to the growth of homoiogenous or heterogenous tumors is distinctly lessened. Reference has been made to the diminution in the intensity of the lymphocytic reaction after homoiotransplantation of normal tissues into the brain. Ebeling (1914) had found indications that in mice which are immune to subcutaneous inoculation of mouse-carcinoma, transplantation into the brain might still be successful. According to the subsequent observation of Shirai, it was possible to transplant mammalian tumors into the brain of a strange species. Murphy likewise noted that heterotransplantation of tumors into the brain may be more successful than transplantation into other parts of the mammalian organisms, and moreover, that the lymphocytic reaction is lacking here provided the transplant has not been in contact with the meninges or with the choroid plexus. Active immunization which was sufficient to prevent tumor growth subcutaneously, was ineffective against a tumor grafted into the brain; but again, a simultaneous transplantation of a piece of spleen tissue into the brain caused the mechanisms of defense against the heterogenous tumor to

become active. E. Harde also observed that heterotransplantation of mouse tumors succeeds better in the brain than in the subcutaneous tissue, but this applies only if nearly related species are used as hosts; transplantation of human tumors into the brain of rodents did not succeed, nor did the transplantation of mouse tumors into the brain of guinea pigs. It seems that in this organ the organismal differentials are less well developed than in most other parts of the body. In this respect the brain behaves somewhat like the testicle, where, according to Gheorgiu, heterogenous tumors can also be transplanted successfully, and even more readily than into the brain. A further favorable site is the anterior chamber of the eye (Smirnova, Greene and Saxton, Greene); however, as to the behavior of the lymphocytes under these conditions, no observations have been recorded so far.

The views of different investigators concerning the role which lymphocytes play in the immunization against transplanted tumors are, to some extent, still contradictory. However, from a review of the results obtained and from our own experiments, we conclude that in the growth of tumors the lymphocytes play a part similar to that which we ascribed to them in the case of grafts of normal tissues. Under certain conditions they may serve as indicators of a discordance between the individuality differentials of host and transplant. If host and transplant belong to different species, polymorphonuclear leucocytes appear around the tumor and invade it, in addition to or instead of the lymphocytes. But, while with normal tissues the accumulation of lymphocytes and their invasive activity may in certain cases become so pronounced that it leads to the destruction of a great part of the transplant, with tumors this effect seems to be much less marked. According to Woglom, the retrogression of tumors is not nécessarily associated with an increased activity of the lymph glands, and in our early work on the retrogression of tumors in homoiogenous organisms, whose individuality differentials differed markedly from those of the hosts, we found no reason to attribute the retrogression to the activity of the lymphocytes; furthermore, Ishii and the writer, in a study of tumors which had been weakened by heat previous to transplantation and which subsequently retrogressed, did not observe an accumulation of lymphocytes around or in the transplant sufficient to account for the retrogression; we did find, however, the formation of a strong connective tissue capsule around such tumors, which may very well have helped to produce an inhibiting effect on the weakened tissue. As in transplanted normal tissue, so also in transplanted tumors the bodyfluids of the host may, to a large extent, determine the fate of the graft, and they may be the principal factor concerned in this effect in the case of tumors.

It is, then, essentially the discrepancy in the individuality differentials of host and transplant which causes the accumulation of lymphocytes, and which may also increase the activity of connective tissue around the transplant; and it is likewise on the basis of a discrepancy between the organismal differentials that immunity develops, which then may perhaps intensify the lymphocytic reaction. If this interpretation is correct, we should expect a distinct accumulation of lymphocytes to be lacking around autotransplants of spontaneous

tumors, and this seems to be the case; it likewise has been found impossible to immunize against a tumor with autogenous tissue. However, there is an experiment by Murphy which does not seem in accordance with this interpretation. He found it possible to prevent the growth of autogenous carcinoma transplants by the local application of erythema-producing X-ray doses to the skin; but in this case we have probably to deal not with an effect on the lymphocytes but with a condition of a different nature. Through radiation the tissue has presumably been made into an unfavorable soil for the development of the graft. The incorporation of the latter into the host and its nourishment are inhibited under these circumstances, which are non-specific and would therefore affect tissues, irrespective of their relationship to the host.

However, lymphocytes are attracted not only locally to a tumor possessing a strange individuality differential, but they are increased also in the general circulation, due to the fact that the homoiodifferentials enter also the blood vessels and various organs. This is another instance of the resemblance of tumors to normal tissues possessing a strong individuality differential. But no proof has been given for the view that it is on account of these lymphocytic changes in the whole organism that immunity develops. The production of antibodies probably depends on the stimulation of the reticulo-endothelial tissue.

The great similarity in the behavior of lymphocytes after transplantation of tumors and of normal tissues, and the significance of the relations of the organismal differentials of host and transplant in determining the nature and intensity of these reactions, has been further confirmed by the recent investigations of Blumenthal, concerning the alterations which take place in the blood cells of animals into which pieces of tumors have been transplanted. There is a complete analogy between such alterations and those which occur after transplantation of normal tissues; differences which do exist are due to secondary conditions. Homoiotransplantation of rat carcinoma and sarcoma into rats brings about an increase in lymphocytes in the blood, which begins on the fourth or fifth day following transplantation and persists for eight to ten days, or sometimes longer, the maximum being reached between the seventh and ninth days. Quantitatively, the reaction is of about the same order as the one following transplantation of homoiogenous normal tissues, and it may occur whether the tumor grows or not. The same results were obtained in mice after homoiotransplantation of tumors, either into the same inbred strain or into strange strains. In these two types of transplantation the changes in the blood were similar, although the tumors grew in the same strain, while they did not grow in strange animals. Still, the increase in the number of lymphocytes in the first type was not quite as rapid as that in the second type, although it tended to persist for a somewhat longer period; in transplantations between different strains, the curve representing the variations in the lymphocyte counts showed a steeper ascent as well as a steeper descent.

Homoiotransplantation of a benign rat tumor, an adenofibroma of the mammary gland, likewise caused an increase in lymphocytes, but it was a little lower than that observed after transplantation of cancerous tissues and there

was a somewhat greater variation as to the time of maximum increase. When in rats and mice with growing homoiotransplanted tumors the period of observation was extended until the tumors had reached a considerable size and the animals had become debilitated, the number of erythrocytes decreased and at the same time the number of polymorphonuclear leucocytes increased in the peripheral blood. A similar parallelism in the changes in erythrocytes and leucocytes occurred in mice with growing autogenous (spontaneous) tumors. There were no significant changes in the number and relative distribution of lymphocytes and polymorphonuclear leucocytes until the tumors became moderately large. From that time on there was a gradual increase in the total white cell count and in the relative number of polymorphonuclear leucocytes. At the same time a decrease in the total number of red cells and an increase in reticulocytes occurred; also, normoblasts appeared in the peripheral blood when the later stages of the anemia were reached. When the tumors became large, the average erythrocyte count fell to 4.12 million cells per cmm. (Blumenthal). In the bone marrow the newformation of the red cells was intensified and it appears probable that the increase in the number of polymorphonuclear leucocytes in the peripheral blood was caused by the stimulation which occurred in the bone marrow as the result of the very marked anemia.

Heterotransplantation of rat and mouse tumors to guinea pig, mouse and rat led in principle to the same changes as those observed after heterotransplantation of normal tissues. There was an increase in polymorphonuclear leucocytes in the peripheral blood, which set in between the second and fourth day after transplantation and which reached a maximum between the sixth and tenth day. It persisted somewhat longer than the increase observed after heterotransplantation of normal tissues, but the degree of increase was about the same in each. In each also the return of the number of polymorphonuclear leucocytes to normal was followed by an increase in lymphocytes, which reached a maximum usually between the sixteenth and eighteenth day after transplantation and then dropped to the usual level. This rise in the number of polymorphonuclear leucocytes following heterotransplantation of tumors and of normal tissues was not associated with anemia and increased erythropoiesis in the bone marrow; it was presumably due to a direct effect of the organismal differentials on the leucocytes or their precursor cells in the bone marrow.

If, twelve or twenty days following the homoiotransplantation of a piece of normal tissue, a second homoiotransplantation of a similar piece is carried out, a lymphocytic reaction follows also this transplantation as well, but in this case the reaction occurs somewhat more rapidly, although the rise is not quite so great in the majority of animals as after the first transplantation. The same effect is obtained if in the first homoiotransplantation a piece of cancerous tissue is used instead of normal tissue and if normal homoiogenous tissue is then transplanted twenty days after the first transplantation. In principle, the same results were obtained if two successive homoiotransplantations of tumors were made; even if the second transplantation was delayed so long

that the first homoiotransplant had elicited an increase in polymorphonuclear leucocytes, a second homoiotransplantation of tumor was again followed by a rise in the lymphocytes.

Furthermore, a like sequence of events was noted when in a first homoiotransplantation normal tissue was used and, sometime later, a piece of homoiogenous tumor. But if instead of homoiogenous tissue, heterogenous tissue was transplanted and this was followed by a second transplant of homoiogenous tumor, the reaction which followed the second transplantation was not modified by the first transplant. It is apparently only a first homoiotransplantation which influences a second homoiotransplantation. The character of the organismal differentials of both the first and second grafts determines the mode of reaction in the blood, but the tissue or organ differential, or the differences between the differentials of normal and tumor tissue belonging to the same speeies, is of no importance as far as this reaction is concerned. These very interesting experiments of Blumenthal eonfirm, therefore, the conclusion that in transplantation of both normal tissues and tumors the organismal differentials are a very important factor in determining the kind of reaction of the host against the graft, and the behavior of the lymphocytes and of the polymorphonuclear leueocytes can be used as an indicator of the relationship between hosts and transplants. It may then be eoncluded that as far as the reactions in the eellular constituents of the blood are concerned, transplants of various kinds of tumors, as well as autogenous tumors, behave like the corresponding transplants of normal tissues, and that the reactions in both instances depend on the relationship of the organismal, and in particular, of the individuality differentials of host and transplant, the differentials of the tumor being essentially the same as those of normal tissues from which they are derived. There is reason for connecting the increase in the number of polymorphonuclear leucocytes in the circulating blood which occurs during a later period in the growth of transplanted as well as of spontaneous autogenous tumors, with the same factors which were responsible for the anemic changes which are noted in the bone marrow.

In the ease of normal tissues we have seen that differences in the constitution of the individuality differentials between host and transplant may cause not only the invasion of the transplant by lymphocytes, but may also induce a more active reaction of the eonnective tissue eells of the host against the graft, and may tend to diminish the ingrowth of capillaries into the transplant. The experiments of Burgess and Tyzzer indicate that also around a graft, whose organismal differential differs markedly from that of the host, connective tissue growth may be quite active, and the resulting increase in the formation of fibrous tissue may still further contribute to the injury of the transplant. But it is exactly the opposite condition, namely, a lack of ingrowth of connective tissue, accompanied by a lack of ingrowth of blood vessels into the graft, a lack of "stroma reaction" on the part of the host tissue, which, according to Russell and Bashford, may result in the destruction of homoiogenous tumor transplants in immunized animals, and they believe the lack of this reaction to be the mechanism through which the active immunity of the

host against the transplant becomes effective. They applied this conception also to animals which had become immune following the retrogression of their tumors. In some way immunization was supposed to interfere with the chemotatic attraction which tumor transplants exerted on the surrounding tissues of the host. Russell and Bashford held that the mechanism of an active immunity, or rather, of an active resistance against transplanted tumors, combined with the apparent lack immune bodies in the bodyfluids of the host, constituted a condition distinct from any other known kind of immunity. On the other hand, natural immunity and active immunity against heterogenous tumors did not depend, in their opinion, upon a lack of stroma reaction, the immunity against heterogenous tumors in particular being due, rather, to the cytolytic effect exerted by the injurious bodyfluids on the peripheral tumor cells, a phenomena related to the formation and action of agglutinins, precipitins and hemolysins which affect certain normal cells or proteins. Similar observations to those of Russell concerning the significance of the stroma reaction, were subsequently reported by Woglom in the immunity against rat tumors, and by Rous in the transplantation of embryonal tissues in mice which had been previously immunized against embryonal mouse tissue. According to Rous and Murphy, in the transplantation of chicken sarcoma into naturally immune fowl, especially into those in which previously retrogression of such a tumor had taken place, the successful inoculation with a second tumor may be prevented either through lack of a stroma reaction or through the subsequent accumulation of lymphocytes. However, later investigations did not confirm the theory of a lack of a stroma reaction as the mechanism underlying the destruction of the grafts in immunized mice (Mottram and Russ, Murphy, Tyzzer and Levin). We and our associates likewise have failed to observe a phenomenon corresponding to it in the case of normal tissues, although Cora Hesselberg and the writer noticed a diminished vascularization of homoiogenous as compared with autogenous grafts.

Russell and Bashford, believing that active immunity depends upon a lack of stroma reaction, assumed that this immunity can manifest itself directly after transplantation only, before the tumor has been incorporated in the host tissue and has begun to grow, However, there is every reason for believing that a retrogression of homoiogenous tumors may be caused by an active immunity which develops in the host during the period of growth of the transplant. In this case there is then an active immunity which does not depend upon a lack of stroma reaction. Moreover, Russell himself noted that in actively immunized animals a small tumor nodule may occasionally grow for some time, but subsequently retrogress. Here, too, the active immunity causing the retrogression does not depend upon the stroma reaction for its manifestation. It is known that under certain conditions homoiogenous and even heterogenous tumors are able to remain alive and even to grow for some time without possessing a stroma. We may then conclude that the lack of a stroma reaction does not play a significant role in the active immunity against homoiogenous tumors.

In a somewhat different way, also, Greene attributes to the stroma a promi-

nent role in the mechanism through which immunity affects the tumor. He believes that immune processes may act on a tumor injuriously by interfering with the formation of the specific stroma which the tumor needs, and that in this way the growth of a carcinoma may be prevented in immune animals. However, it is much more probable that in his experiments the immune processes acted primarily on the tumor cells directly, diminishing their growth energy, and that as a result of this interference the relations between the tumor parenchyma and the ingrowing connective tissue were changed. We have found in other instances definite correlations between the parenchyma and stroma, in which the condition of the former was the primary and decisive factor which determined the condition of the latter.

In the case of microorganisms there is good reason for assuming that the reticulo-endothelial tissue is the seat of the production of immune bodies, and there are strong indications that also the immunity against tumor transplants, as far as it is caused by differences in the organismal differentials between host and transplant, is due to a stimulation of the reticulo-endothelial system; the activity of the lymphocytes and polymorphonuclear leucocytes are apparently factors of secondary importance in the mechanism underlying this immunity; they function mainly as indicators of the relationship between the organismal differentials of host and transplant. Various investigators, Apolant, Uhlenhuth, Vorländer, Caspari, have assumed that it is the reticulo-endothelial system which gives origin to tissue immunity. In the reticulo-endothelial tissues abnormal cells or strange colloidal substances circulating in the bodyfluids are held back and phagocytosed, and here, especially in the spleen and bone marrow, they set in motion the mechanisms leading to the production of immunity. The main evidence for the conclusion that this applies also to immunity against transplanted tumors consists in the demonstration that different types of this immunity, such as concomitant and retrogression immunity, and probably also certain instances of natural immunity, can be abolished by inactivation (blockade) of the reticulo-endothelial system, either by injection of substances such as India ink, colloidal metals or dyes (Roskin, Lignac and van de Borne), or by means of strong doses of X-rays. Weak doses of X-rays, or certain other procedures, such as stimulation of the spleen through ultraviolet rays (Roskin), may stimulate the reticulo-endothelial cells and thus produce an opposite effect, leading to an increase in immunity against tumor transplants. There is the possibility that in addition to the formation of the immune substances the reticulo-endothelial cells may be concerned in the production of the primary preformed substances circulating in the bodyfluids which act on organismal differentials, although such a function has not yet been demonstrated. Quite recently Ehrich and Harris have found evidence that also local lymph glands may participate in the production of antibacterial immune substances and of immune hemolysins and they noted that such lymph glands show a hyperplasia of the lymphocytic tissue. This observation suggests the possibility that it may be the lymphocytes rather than the reticulo-endothelial cells which produce these substances. We would then have to assume that the lymphocytes react to strange differentials in a twofold way, namely by movements and by the production in immune substances.

We have discussed those aspects of immunity against transplanted tumors, in which organismal differentials function as antigens. It may be further stated that the organismal differentials in tumors, are essentially the same as those of the normal tissues from which the tumors are derived. There are, however, indications that other substances present in tumors, besides the organismal differentials, may be antigenic. A brief outline of some of the principal data which point to the presence of these secondary antigens will now be given.

(7) *The presence of antigens other than organismal differentials in tumor cells.* By serological tests the same types of antigenic constituents have been found in certain cancers, which normal cells in corresponding organs of the same species possess, namely, species-specific, organ-specific, blood-group and heterophilic Forssman antigens; also alcohol soluble substances corresponding to Wassermann antigens not characteristic of either organ or species may occur. Thus the cells of a carcinoma developing in individuals belonging to blood group A may contain these same blood-group antigens and the partial Forssman antigens which are associated with blood group A. Mouse carcinoma may contain Forssman antigen, in accordance with the fact that the mouse belongs to the group of those species which possess heterophilic antigens. However, there are apparently some exceptions to this parallelism between normal tissues and tumors. According to Kritchewski and Rubinstein, also the Flexner-Jobling rat tumor contains Forssman antigens, although normal rat organs do not contain them; this would constitute a difference between cancerous and the corresponding normal tissues. Moreover, while human carcinoma cells of individuals belonging to blood group II, like some normal cells, may possess the A differential, it has been stated that it is never found in sarcoma cells; but it is not certain that normal connective tissue cells of such individuals contain it. Hence we find in cancer cells a complex condition, which makes the search for constituents characteristic of carcinomatous cells, and not present in normal cells, difficult, and this may explain at least in part the contradictory nature of some of the results obtained by various investigators. Some of these obstacles to the discovery of specific tumor antigens were overcome by using for immunization carcinomatous material from persons belonging to blood group I, which is free of antigens A and B, or by first extracting the blood group antibodies by means of erythrocytes possessing group A. Furthermore, the attempt was made to modify the material to be used for immunization by destroying or eliminating the species and normal organ antigens before injecting the antigen. Hirszfeld and his collaborators found that by immunizing rabbits with human carcinoma of a certain organ, immune sera developed in a small minority of the rabbits which reacted with different types of human carcinomas, irrespective of the organs in which they originated. Witebsky and Lehmann-Facius, by using boiled, instead of fresh, unheated carcinoma suspensions as antigens, obtained antibodies which were specific for carcinoma, and not merely for the species or the organ in which the cancer occurred. Witebsky, in addition, used boiled globulins of cancer tissue in these tests. According to Lehmann-Facius, the complement fixation

which takes place when antigen and immune substances interact, can be made still more specific if the test is carried out at a temperature near the freezing point. But, while Witebsky assumes that the immune serum obtained against the carcinoma of a certain organ as a rule reacts specifically with the antigens prepared from a carcinoma of the same organ, and only exceptionally with cancers from other organs, Lehmann-Facius maintains that the antisera show a positive complement fixation reaction with all kinds of human cancers, and even with cancers of other species.

But, in addition, Witebsky and Morelli found that if in rabbits immune substances are produced against human sarcoma, these immune substances react with alcohol extracts not only of human sarcoma, but also of carcinoma and even of various normal human organs; but these substances are species-specific and do not react with rat or chicken sarcoma. However, it is possible to absorb from such immune serum the quota reacting with normal organs and to leave behind the anti-tumor fraction; also the anti-carcinoma fraction can be specifically absorbed with carcinoma extract, so that in the end only the sarcoma antibody is left in the immune serum. It seems, then, from these and other experiments, that antibodies can be produced against constituents which are specific for cancers in general, but that antigenic differences exist between the various kinds of tumors. These antibodies, and therefore also the antigens producing them, may or may not carry species differentials. However, somewhat different are the more recent results of Lehmann-Facius (1932), who finds that ether extracts of mouse intestines may induce the formation of antisera, which react with cancer extracts of a lipoid nature; lipoid antigens which are present in cancer do not need, therefore, to be specific for the latter, but may occur also in normal organs.

It is possible to obtain anti-cancer sera under conditions in which species-specific immune bodies do not develop, as for instance, when boiled antigens are injected; but such methods do not exclude the production of organ-specific immune sera. Some of these observations suggest that the antibodies which are common to various anti-carcinoma sera are organ-specific constituents, and that, correspondingly, the change in the constitution of normal cells, which leads to their transformation into cancer cells, represents a change in the organ rather than in the organismal differentials, which latter seem to be essentially the same in cancer and in normal cells. Accordingly, Witebsky's method of injecting cancer globulins for preparing immune sera against cancer, can be readily used also for the preparation of organ-specific immune sera if, instead of cancer globulins, globulins from various organs are taken as antigens.

But, there are still further differences between antigens obtained from cancer and from normal tissues. According to Witebsky, in carcinoma cells the lipoids may exist in a state which makes them readily available as antigens, while in normal organs they are less available, perhaps because they are bound to other cell constituents.

There are some additional indications of the presence of specific cancer antigens. Hirszfeld and Halber, assuming that there are immune substances

against cancer antigens in the serum of cancer patients, mixed alcohol extracts from a human carcinoma with the blood serum of patients suspected of cancer and observed a specific complement fixation. A positive reaction was obtained irrespective of the organ in which the carcinoma had arisen. However, other investigators consider this test as non-specific, or at best, as successful in only a small number of instances. Lehmann-Facius, in ordei to demonstrate the presence of specific immune substances in the serum of cancer patients, used the euglobulin fraction of the serum and the phosphatid fraction of tumor extracts. Also, the diagnostic cancer reactions of Freund and Kaminer, of Willheim and Stern, and of Fuchs, are based on the assumption that in the serum of cancer patients substances are circulating which are specific for all kinds of human cancer, which develop in response to antigens characteristic of cancer, and which interact with these antigens in a specific manner. The substances present in the cancer sera may be either proteolytic or lipolytic. Moreover, not only human cancers, but also animal cancers, may contain these antigens. But there is still some difference of opinion as to the degree of specificity attaching to these various tests.

We have already referred to the experiments of Lumsden, who believes that besides preformed natural and experimentally produced immune substances which are species-specific, there exist in the serum constituents which act in a specific manner on various kinds of cancer cells growing in vitro, although they are able to injure, also, reticulo-endothelial cells growing out from pieces of spleen in tissue culture. As stated, it is not certain at present whether these reactions are due to the species differentials present in cancer and in spleen tissue, or whether they are due to "antimalignancy" antigens, calling forth the production of the corresponding antibodies. On the whole, the evidence seems to point to the conclusion that the reactions which Lumsden observed were caused by species differentials, therefore, by organismal differentials, and not by a special kind of antigen, designated by this investigator as "antimalignancy" antigens. More recently, Mann and Welker produced in rabbits, injected with preparations from various types of human cancer, antisera which contained precipitins for the proteins present in autolysed carcinoma, but not as a rule for proteins from normal human tissues; these immune substances reacted also with the blood serum from cancer patients, and most strongly with the serum of patients that were bearers of the same kind of carcinoma as the one from which the antigens that were used in the preparation of the precipitins had been obtained. This suggests that the specific carcinomatous proteins are present also in the blood of cancer patients. Such serum is species-specific; it reacts only with human sera, not with those of various animal species. These precipitins were therefore, specific for protein from carcinoma and at the same time they were species-specific.

There is, furthermore, noticeable in some of the diagnostic tests for cancer mentioned above, a similarity between the reactions of cancer sera and of embryonal or pregnancy sera; embryonal cells may be affected by these sera in a similar manner to cancer cells, and in embryonal tissue, antigens similar to cancer antigens may be present. Moreover, Hirszfeld and Dmochowski

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There are some additional indications of the presence of specific cancer antigens. Hirszfeld and Halber, assumiog that there are immune substances

cifically on the transplanted leucocytes, might be responsible for the peculiarities noted.

There exist, then, tumors in which an extraneous growth stimulus (virus) determines the reaction of the host against the transplant and the specificity of the immune sera. In other cases the reaction may be determined by growth stimuli intrinsic in the cancerous cells (Gi). These stimuli may perhaps be similar to factors active in embryonal tissue, although in some very essential respects conditions prevailing in tumors and embryonal tissues differ. In still other instances, perhaps, substances related to tissue and organ differentials, such as those present in pus and necrotic tissue, may give rise to immune substances. However, definite data as to the nature of some of the antigenic substances found in these cancers, and also in leukemia, which would differentiate them from normal tissues, are as yet lacking. Moreover, some characteristics of tumors may change in the course of serial transplantations; we have discussed the probable nature of these changes in a preceding chapter. Dmochowski, in the case of some mammalian tumors, found indications that also the antigens may change as the result of long-continued serial transplantations. Similar are the recent observations of MacDowell and his associates in mouse leukemia. Mice belonging to strain C58 develop leukemia in a very large percentage of cases. Leukemic cells from a C58 animal with spontaneous leukemia can readily be transplanted into other C58 mice, where they proliferate and so transfer their disease to the hosts. When several lines of leukemic cells were propagated through a large number of generations of C58 mice, in the course of these passages the cells gained in proliferative power and transmitted the disease more readily to other C58 mice. MacDowell found that, through graded inoculation with increasing doses of such leukemic cells, C58 mice can be immunized against these various propagated lines, so that in the end the transfer of such cells no longer calls forth leukemia in mice thus treated. But, if a C58 mouse, immunized against these special lines of C58 leukemic cells, is inoculated with cells taken directly from a case of leukemia arising spontaneously in a C58 mouse, then the inoculated mouse succumbs to the disease. The immunization with the serially propagated leukemic cells from strain C58 protects only against these special propagated lines, but not against new leukemic cells which have not yet been propagated in passages. It appears therefore, as if, as a result of the serial propagation, not only did the leukemic cells acquire a greater growth energy and become therefore more virulent, but there must also have taken place in all the serially propagated lines the same type of modification of the antigen, which made this antigen different from that present in the leukemic cells from primary spontaneous cases. As to the nature of this antigen, it may represent an intrinsic stimulus or an extrinsic virus, or something akin to a tissue or organ differential; but for the reasons stated, it is not probable that this antigen or any of the special tumor antigens originate as a result of somatic mutations occurring in the cells. There occurs then, in cancer cells, in addition to the organismal differentials, various other kinds of antigens, but there remains some doubt at the present time as to the nature of some of these antigens of the second type.

have shown that spontaneous as well as long-transplanted tumors, and also rapidly growing embryonal tissues, contain antigens which are also present in dying leucocytes (pus) and in necrotic tissue. Antigens which are common to long-transplanted tumors and pus are species-specific; for instance, the Brown-Pearce rabbit carcinoma has an antigen in common with rabbit pus, and the guinea pig liposarcoma has an antigen in common with guinea pig pus.

We have already mentioned the fact that in the sera of animals which bear virus-induced cancers antibodies may be demonstrable, which are directed against the virus. Such sera may contain substances which neutralize the virus, or in combining with a virus antigen, call forth complement fixation or lead to the formation of precipitates. Thus the filtrable Rous fowl sarcoma contains virus antigen which may induce in the blood of geese, injected with the tumor extract, antibodies against this virus (Rous, Robertson and Oliver). Similar immune substances may be demonstrated in the serum of chickens in which such tumors have retrogressed (Rous, Mottram). They may also develop in bearers of slowly growing tumors of this kind (Andrewes) and one kind of tumor may elicit the production of antibodies which interact also with the virus of a different type of fowl sarcoma. According to Peyton Rous and Kidd, the growth of the rabbit papilloma, which is caused by a virus, as well as carcinoma which develops from this papilloma, may give rise to specific immune substances circulating in the serum of the bearer of this newformation. In saline extracts of the cottontail rabbit papillomas, a serologically specific substance can be demonstrated, which probably is identical with the Shope papilloma virus. It is of special importance that, according to Rous, if a papilloma virus-induced carcinoma develops in domestic rabbits, the virus is no longer demonstrable in the tumor extracts; but the presence of such a virus can be made very probable, because specific antibodies against the papilloma virus are demonstrable in the blood serum of these rabbits. Similarly, Andrewes has shown that when sarcoma is produced in fowl by injection of carcinogenic substances and a filtrable agent cannot be shown to exist in this tumor by direct methods, the presence of a hidden agent in the tumor may be made probable by means of immune substances which can be shown to exist in the blood serum of the fowl. Kidd found, with the aid of the complement fixation method, an antigen in the extract of the Brown-Pearce rabbit carcinoma; this antigen was not of the same type as the antigens present in normal rabbit organs, nor was such an antigen noted in tumors occurring in the uterus of the rabbit; but this antigen was also distinct from the rabbit papilloma antigen and the presence of a virus has not so far been demonstrated in the Brown-Pearce rabbit carcinoma.

In this connection it may also be of interest to recall the experiments of Furth on transplantation of leukemic cells from a case of leukosis which arose in F_1 hybrids between two strains of mice differing markedly in their tendency to become leukemic. The results of transplantations from F_1 hybrids to both parent strains could not be interpreted merely as due to differences in the relationships of the individuality differentials of the two parent strains and the hybrids. We suggested that exogenous growth stimuli (Ge), acting spe-

against tumors retained distinctive features and there was the expectation and hope that a study of immunity against transplanted tumors might lead to the discovery of methods of immunization also against spontaneous tumors, which would prevent their development or cause the retrogression of tumors which had already developed. Similar views were held, also, by subsequent investigators, Tyzzer, Woglom, Uhlenhuth, Chambers and Scott, Caspari, Lewin and Lumsden.

We approached the problem of tumor transplantation essentially from the point of view of the experimental analysis of tissue growth, and from the beginning we emphasized the parallelism between the behavior of tumors and normal tissues after transplantation. The favorable results of autotransplantation were attributed to the similarity in the constitution of host and spontaneous tumor, and the reactions in homoiotransplantation, and the still stronger reactions in heterotransplantation, were correspondingly interpreted as due to the relative strangeness of the constitution of host and transplanted tumor. In this sense we explained also the experimentally produced variations in growth energy of tumor cells which we had observed under various conditions, and the conclusion was drawn by us that the potential immortality of tumor cells which the serial transplantation of tumors had revealed was not peculiar to tumors, but was shared by the majority of normal tissues, at least by all of those that could give origin to tumors. A comparison of the structural changes of normal tissues and tumors after transplantation and of cellular reactions taking place around them revealed additional similarities, and it was possible to distinguish between the constitutional factors, which would permit the tumor cells to live in the host, and the increased proliferative tendency inherent in the tumor cells, which enabled them to grow after transplantation. A distinction was made also between transplantability and the factors determining the growth energy of tumors and at the same time the analysis of the constitutional factors underlying these conditions was further developed. There was noticeable a great similarity in the behavior of normal tissues and tumors after auto-, homoio- and heterotransplantation.

These points of view were extended by Peyton Rous, who (1910) compared the immunity against embryonal tissues and against tumor tissues, when both tissues grew side by side in the same host. He found that immunization against embryonal tissue, and that against tumor tissue, took a similar course. A few years later (1916) we further compared the reactions of the host against transplanted normal and tumor tissue, and we observed in both instances a parallel reaction of the lymphocytes and connective tissue of the host against the transplant. Thus there was formed gradually on the basis of these comparative studies of tissue and tumor transplantation, the concept of organismal differentials as the essential factors underlying both of these processes.

A definite divergence existed, therefore, between these two tendencies in the development of cancer research, and especially in the study of the transplantation of tumors; in the one, the immunity against tumors was the central problem, in the other, it was the comparative behavior of normal and tumor tissues. However, this distinction was not quite so complete as it might appear. Thus,

Chapter 5

Tumor Growth and Organismal Differentials

In the preceding chapters the principal facts concerning the significance of organismal differentials for the growth of transplanted tumors have been analyzed. The concept of organismal differentials has contributed in various ways to the understanding of tumor transplantation and of the immunity against transplanted tumors; and conversely, the analysis of tumor growth has contributed to the understanding of the organismal differentials. It is for these reasons that we have discussed also the various factors which interact with the organismal differentials in tumor growth. In concluding, it will be of interest to trace the development of the various concepts and theories relating to the factors which are of importance in the transplantation of tumors. Some of the most prominent investigators in the field of cancer have contributed to these studies, and while certain of their interpretations have been modified in the course of time, the conclusions they expressed and the experiments they carried out in support of them helped greatly to advance our knowledge of the nature of cancer and of the factors active in transplantation.

Jensen in his transplantations of mouse carcinoma approached the facts be discovered from the point of view of the bacteriologist and immunologist. It had been found possible to induce immunity against various diseases caused by microorganisms. By using as a vaccine, in a weakeoed form or in a very small quantity, the microorganisms that caused the disease or certain of their derivatives, or by introducing related organisms less virulent for the host but sufficiently related to the causative agent, an active immunity was produced. These studies gave direction to and supplied the problems for Jensen's work as well as for the following investigations of Ehrlich and Apolant, and also of Bashford, Murray, Haaland, Russell and Cramer. In the beginning it was assumed that cancer cells differ in various ways from ordinary tissue cells and that the laws relating to the transplantation of tumors differ in some essential respects from those governing ordinary tissue cells. Thus Ehrlich applied the same principles in explaining immunity against cancer and immunity against microorganisms; he explained both on the basis of his nutriceptor and athrepsia concepts. However, Bashford and his associates, Murray, Russell and Cramer, soon recognized important differences between these two types of immunity, and one of the most essential was the fact that a formation of antibodies against the ordinary transplantable tumor could not be demonstrated in the case of tumor immunity; they substituted therefore the term "resistance" for that of "immunity." But even these investigators considered the problem of immunity or induced resistance against tumor growth as the principal problem of tumor growth.

Although soon some facts were established, which proved certain similarities between the behavior of tumors and of normal tissues, still the immunity

in fixing the number of genes for this purpose, he assumed that these factors were specific determinants of tumor immunity, and that they did not apply to tissues in general.

These differences in the theories of various investigators are shown more clearly in additional investigations. Jensen (1908-1909) compared tumors growing after transplantation into other individuals with metastases of spontaneous tumors, an interpretation subsequently expressed by various other authors. He believed, furthermore, that if a change in diet can affect transplantability of tumors—as it apparently did in Haaland's experiments—it might equally influence metastasis formation. No distinction is recognized here between the conditions in auto- and in homoiotransplantation. On the other hand, Bashford, Murray and Cramer made a sharp distinction between the conditions that cause the formation of a spontaneous tumor and those determining the growth of a tumor once it has formed; they were led to this distinction by the observation that an animal unsuccessfully inoculated with a transplantable homoiogenous tumor, subsequently could develop a spontaneous tumor. They did not, however, distinguish in these cases between the development of a spontaneous tumor possessing the same or almost the same individuality differentials as the host and the growth of homoiogenous (transplanted) tumors, in which the individuality differentials of tumor and host differ; this can be seen from their statement that they observed—evidently contrary to their expectations—that animals affected by spontaneous cancers are not greatly more susceptible to inoculation with cancerous tissue than are normal animals. They believed that spontaneous tumors which do not grow in other animals of the same species not affected by cancerous growth, rarely grow when transplanted to other parts of the animal's own body, and not at all in other animals bearing spontaneous tumors; there was no need, therefore, for any subsidiary assumption as to the importance of a constitutional condition inherent in the normal cells of the animal in which the tumor originated and in the fully developed tumor cells for the growth of spontaneous cancer after transplantation. What these authors called "individuality" of tumors was not the chemical constitution of the tumor cells as determined by genetic factors; identity of individuality did not mean identity in chemical composition of cells due to genetic factors, but it was considered to be the result of identity of the sum total of changes which had taken place in the tumor cells, in consequence of past experiences in the life of the organism. According to this conception, every individual mouse was therefore different from all the others, and this difference would increase with the increasing length of life of the animal. This point of view is expressed in a paper by Bashford, Murray and Cramer on the resistance of mice to the growth of cancer (1907).

Bashford and his collaborators attributed differences in transplantability of tumors to factors inherent in the host as well as to variable factors which distinguish different tumors. Among the latter they also recognized the significance of the growth energy of the tumor, and they insisted especially on the great significance of growth rhythms in tumor cells which occur spon-

in our early analysis of tumor transplantation we admitted the possibility that extraneous agents might be concerned in the growth of tumors, and this suggested a possible difference in the behavior of normal tissues and of tumors. Our findings regarding the difference between the results of auto- and homoiotransplantation of tumors, the observations of Schoene and Bashford that an immunity against tumors could be produced by inoculation of normal tissues, but that it was *impossible to immunize* with autogenous tissues (Apolant, Woglom), as well as the behavior of heterotransplanted tumors, suggested also to Ehrlich and Bashford that in tumor immunity there may be a component directed against the tumor as a tissue and not as a tumor. Ehrlich even went so far as to state that tumors showed in this respect a greater specificity than normal tissues, inasmuch as he assumed that the latter could be successfully transplanted between individuals belonging to different but hybridizable varieties, while it was difficult to transplant tumors even to different strains within the same species; and Bashford, Murray and Cramer interpreted the condition following retrogression of a tumor, which was designated as panimmunity by Ehrlich, not as due to a specific tumor immunity but as directed against the tissues of which the tumor was composed. In a similar manner Bashford and Russell (1910) explained the immunity produced against a second heterogenous tumor through a first inoculation with the same heterogenous tumor; in this case, too, they assumed that the immunity was directed not against the tumor but against the tissues. Some years later Murphy transplanted not only heterogenous tumors, but also heterogenous embryonal tissue, into the chick allantois and found that both tumor and embryonal cells behaved similarly under these conditions, although he stressed the results obtained with tumors rather than those obtained with embryonal tissues. Little also, in 1922, using more closely inbred strains of mice, compared the genetic factors underlying the transplantation of tumor tissues with those effective in the transplantation of normal spleen from points of view similar to our own.

Yet notwithstanding these analogies between the growth of tumors and normal tissues, which began to accumulate more and more, the large majority of authors still conceived tumor growth and tumor immunity as essentially distinct from the growth and immunity of normal tissues. This was true, as mentioned, of Ehrlich as well as of Bashford and his associates. The latter saw one of the characteristic features of tumor immunity in the lack of stroma reaction, as first defined by Russell. Moreover, they attributed all the reactions of the host against tumors to an induced active immunity against tumors (Russell), in contradistinction to the writer's subsequently defined concept of preformed individuality differentials, to which the primary reaction against the homoiogenous transplant, in the case of normal as well as of tumor tissues, was attributed; also, the active immunity against tumors was considered by us as resulting from differences in organismal differentials between host and tumor. Tyzzer, who recognized the importance of hereditary constitutional factors in the immunity against tumors, likewise accepted Russell's interpretation; in estimating the factors which determine the transplantability of tumors and

also the more recent work of Lumsden concerning the adaptation of tumors to the action of heterogenous serum, resulting from a temporary growth in a strange species, was in conformity with this view. While there is much evidence for the conclusion that tumor cells may display a remarkable ability of adaptation to new environments, the transplantability of tumors is determined above all by the relation of their organismal differentials to those of their hosts. Haaland and Woglom were struck by the observation that in the same individual one tumor, a spontaneous cancer, may continue to grow, while another, a transplanted tumor, retrogresses. However, such an occurrence is to be expected if we consider the great similarity or identity of the individuality differentials in the host tissues and in the spontaneous tumors and their differences from those of the strange transplanted tumors. The importance of the relation between the individuality differentials of host and transplant had not yet been recognized by Haaland, who attributed the difference in the fate of the two tumors to local conditions residing in the tumor cells. Indeed, the sharp distinction between autogenous and homoiogenous tumors which the theory of the individuality differentials implies had not yet been made by the majority of authors. Thus as late as 1916, Tyzzer applied the findings concerning the growth of homoiogenous tumors to the explanation of the origin of spontaneous tumors. He compared the lack of development of a spontaneous tumor with the non-take of a homoiogenous tumor and defined the factors which prevent a spontaneous tumor from developing or from expanding as immunity; the means for regulating the growth of autogenous tissues were considered analogous to those which determine immunity against transplanted tumors. He further concluded that spontaneous tumors must have feeble antigenic power and offer effective resistance to the normal influences which inhibit undue tissue growth; in this way the continuous growth of a tumor is made possible in the animal in which it originates. Otherwise reactions sufficient to destroy spontaneous cancerous growths would occur more frequently. A spontaneous tumor, according to this investigator, is therefore a parasite strange to the host and it owes its origin to a somatic mutation. Similarly, L. C. Strong and his associates expressed the opinion that a genetic analysis of the factors underlying tumor transplantation will explain also the origin of spontaneous tumors. Inasmuch as according to these authors it is possible by means of transplantation to determine the specific number of factors (genes) which each tumor requires for its growth in a strange host, it was perhaps tacitly assumed that the number and character of these genes explain also the development and peculiarities of a spontaneous tumor.

However, it follows from the concept of organismal differentials that an analysis of the factors underlying transplantability of tumors can give an insight only into the difference between the genetic constitution of the host and the tumor graft, and that there is no reason for assuming that the hereditary conditions which favor the development of a spontaneous tumor are identical with the genetic factors which would be required for the growth of a transplanted tumor, when these factors are determined according to the procedure

taneously and in which periods of depression and of great intensity of growth alternate; tumor cells which are in the phase of depression offer great difficulty to transplantation. There were two facts known at that time which demonstrated the dependence of the fate of the transplant on factors which are present also in normal tissues, namely, the difference in the results of auto- and homoiotransplantation of tumors, and the species-specificity of the antigens in normal tissues which immunized a host against the growth of a transplant, the latter indicating a parallelism between immune reactions against tumors and against normal tissues and their proteins. Bashford, Murray and Cramer pointed out the species-specific factors in the production of immunity against tumors and in the production of hemolysins and precipitins. Yet, they considered the immunity against transplanted homoiogenous tumors as due to a lack of the stroma reaction, a special phenomenon not heretofore described in the transplantation of normal tissues. Moreover, as stated, they did not attribute the specificity of the tumor tissue to its genetic constitution, but to environmental factors which induce processes of adaptation in the tumor to the organism in which it develops. These investigators conclude that the "influence of individuality, i.e., the sum total of changes due to the past life of the organism, will be to make any mouse different from all the others and these differences will increase the longer the animal lives." In the new host the environment is so strange that the cells cannot survive the interruption of their nutrition. Their failure to grow does not necessarily imply that they would fail to proliferate in their new hosts if the conditions to which they had been accustomed would be immediately supplied in the experiment. "Cells which have lived and have become accustomed to the bodyfluids of one mouse for, say, two years, may easily die or fail to adapt themselves when transferred to the bodies of new animals." Autogenous tissues would then differ from homoiogenous tissues in that the former have had a chance to adapt themselves to the bodyfluids of the host, while homoiogenous tissues have not had such an opportunity. It is evident that this conception differs in some very essential respects from the conception of the organismal differentials. The organismal differentials are the derivatives, the phenotypic manifestations of the genetic constitution of the fertilized germ cells and of the tissues. The organismal differentials in host and transplant and their mutual relationship represent the constitutional factors which determine transplantability of normal tissues and also of tumors; other factors also enter into this process.

Bashford, Haaland, Woglom, and more recently, Lumsden, attributed therefore the transplantability of a tumor, in the main, to secondary processes of adaptation which take place between the tumor and the host in which it originated, or into which it had been transplanted. The origin as well as the transplantability of tumors would therefore depend upon variable, fluctuating factors. It is especially the older experiments of Haaland which suggested this point of view. Haaland believed that he had shown the apparent influence of environmental, and especially of nutritional, conditions on the character of the animals and their ability to serve as hosts of transplanted tumors. But

also the more recent work of Lumsden concerning the adaptation of tumors to the action of heterogenous serum, resulting from a temporary growth in a strange species, was in conformity with this view. While there is much evidence for the conclusion that tumor cells may display a remarkable ability of adaptation to new environments, the transplantability of tumors is determined above all by the relation of their organismal differentials to those of their hosts. Haaland and Woglom were struck by the observation that in the same individual one tumor, a spontaneous cancer, may continue to grow, while another, a transplanted tumor, retrogresses. However, such an occurrence is to be expected if we consider the great similarity or identity of the individuality differentials in the host tissues and in the spontaneous tumors and their differences from those of the strange transplanted tumors. The importance of the relation between the individuality differentials of host and transplant had not yet been recognized by Haaland, who attributed the difference in the fate of the two tumors to local conditions residing in the tumor cells. Indeed, the sharp distinction between autogenous and homoiogenous tumors which the theory of the individuality differentials implies had not yet been made by the majority of authors. Thus as late as 1916, Tyzzer applied the findings concerning the growth of homoiogenous tumors to the explanation of the origin of spontaneous tumors. He compared the lack of development of a spontaneous tumor with the non-take of a homoiogenous tumor and defined the factors which prevent a spontaneous tumor from developing or from expanding as immunity; the means for regulating the growth of autogenous tissues were considered analogous to those which determine immunity against transplanted tumors. He further concluded that spontaneous tumors must have feeble antigenic power and offer effective resistance to the normal influences which inhibit undue tissue growth; in this way the continuous growth of a tumor is made possible in the animal in which it originates. Otherwise reactions sufficient to destroy spontaneous cancerous growths would occur more frequently. A spontaneous tumor, according to this investigator, is therefore a parasite strange to the host and it owes its origin to a somatic mutation. Similarly, L. C. Strong and his associates expressed the opinion that a genetic analysis of the factors underlying tumor transplantation will explain also the origin of spontaneous tumors. Inasmuch as according to these authors it is possible by means of transplantation to determine the specific number of factors (genes) which each tumor requires for its growth in a strange host, it was perhaps tacitly assumed that the number and character of these genes explain also the development and peculiarities of a spontaneous tumor.

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used by Tyzzer, Little and Strong. Also, Uhlenhuth, who recognized the species-specific characteristics of tumors, has not apparently considered the relations between the individuality differentials of the tumor and the tissues of the host. He explained the low degree of immunity against spontaneous tumors by the assumption that the defense mechanism against parenterally introduced cells may not be of a high degree of efficiency. Therefore, pieces of spontaneous tumors, even if they possess only a low virulence, can be reinoculated into the animal in which they originate. Likewise, Caspari assumed that the factors, in particular necro-hormones, which induce immunity against homoiogenous tumors, would be equally effective in the case of autogenous spontaneous tumors. Chambers and Scott (1924) regarded immunity against cancer as analogous to immunity against bacteria. They believed a substance is given off during the early stages of autolysis of tumor cells, which acts as antigen and elicits immunity against the cancerous tssue. The reason why spontaneous tumors, and especially human spontaneous tumors, do not call forth immunity in the bearer, in contrast to transplanted tumors, is that in spontaneous tumors the cells are healthy, the implication being that for this reason they do not give off the immunizing substances; yet, there can be no doubt that autolysing and necrotic areas are frequently found also in spontaneous tumors. Similar views were expressed also by Woglom (1919). The ready growth of spontaneous tumors in the animals in which they originate is due to an adaptation which has taken place between the tumor cells and the bodyfluids; but there is a general resistance against the growth of spontaneous tumors as well as against transplanted tumors (1923), and furthermore, it needs to be explained why resistance cannot be established in all transplanted tumors (1922). C. Lewin assumed that during the development of a spontaneous tumor, which means, during the transformation of normal tissue cells into tumor cells, the former lose the characteristics which make them constituent parts of the host organism; they behave like foreign cells. He therefore concluded that it should be possible to elicit an immunity reaction against a spontaneous, as well as against a transplanted tumor. A cure of a spontaneous tumor would depend therefore on conditions similar to those which determine the retrogression of a transplanted tumor.

This analysis shows the difference between the views which have been expressed by some of the most prominent investigators concerning the distinguishing features of tumor growth, the relations between the factors which determine the growth of transplanted tumors and the origin and further growth of spontaneous tumors; it also presents the interpretations which have gradually developed in conformity with the theory of the organismal differentials. These views are based on some of the earlier observations on tumor growth, which we have discussed already, and on a comparison of the fate of transplanted normal and tumor tissues. All these experiments, as well as those of Tyzzer and Little, and especially the extensive investigations of Little and Strong and their associates on the transplantation of tumors in closely inbred strains, showed the importance of genetic factors in tumor transplantations. Nevertheless, certain differences have developed between

the theory of organismal differentials, based on the comparative studies of transplantation of normal tissues and of tumors, and the concepts of Little and Strong. These authors, did not determine differences which existed between the individuality differentials of tumors and their hosts, but they dealt instead with certain factors which they believed were needed in a specific manner for the growth of transplanted tumors.

As we have shown in the foregoing pages, there is good reason for assuming that the problem of the transplantability of tumors is complicated by a number of variable factors, including changes in growth energy of the tumor, adaptation of the tumor cells to the hosts, different degrees of sensitiveness of different tissues or cells to injurious conditions, and lastly, processes of immunity, which again depend upon complex conditions, such as the ability of the tumor to give off antigens and to absorb and neutralize antibodies. While genetic factors enter also into these conditions in conformity with the fact that the organismal differentials may act as antigens and that the range of reactivity of an organism to environmental conditions is limited by constitutional factors, still, the fact that also external factors are involved in these processes makes it impossible to account for the transplantability of tumors entirely on the basis of Mendelian heredity, and to refer modifications of transplantability entirely to genetic mutations, either in the host or in the tumor cells themselves. These difficulties have been discussed in the preceding chapters.

As to the relations between the origin of spontaneous tumors and the fate of transplanted tumors, it is certain that tumor cells even more than regenerating cells have properties which differ from those of normal cells. As the result of acquired characteristics, tumor cells may be more readily accessible to certain injuries than some types of normal cells. Various physical and chemical agencies affect the former somewhat differently from the latter, but the changes which have taken place during the cancerous transformation are in all probability not specific, in the sense that they depend upon alterations in the constitution of the organismal differentials of the affected tissues, as the result of which it would be possible for antibodies to develop against the individuality differentials of the tumor cells in the host in which they originate.

It is especially the recent investigations of Blumenthal, in which the effects of transplanted normal tissues and transplanted tumors on the distribution of the leucocytes in the circulating blood were compared, which again confirmed in a very convincing manner the essential similarity in the principles underlying the transplantation of both normal and tumor tissues, and which again demonstrated the fact that in both instances it is the nature of the organismal differentials in the host and transplant which primarily determines the outcome of these transplantations. However, in addition to the organismal differentials which normal tissues and tumors have in common, there occur other substances which are also the same in both and which likewise may function as antigens, and lastly there are at least indications that various types of tumors may possess specific antigenic substances which distinguish them from normal tissues.

Organismal differentials and in particular individuality differentials are

then the same in tumors and in the normal tissues in which the tumors originate and these differentials are among those substances which may function as antigens and call forth immune processes against transplanted tumors. This interpretation is not invalidated by the recent experiments of L. Gross in which it has been shown that, if in the inbred strain C3H after transplantation of a tumor originating in another mouse belonging to this strain, this tumor undergoes complete regression, after a preliminary period of growth, the inoculated mouse has acquired thereby, at least in a number of cases, an immunity against a second transplanted tumor of the same type.

Inasmuch as Gross assumes in accordance with the widely prevalent opinion that in strain C3H all individuals are genetically homozygous, he concludes that tissue constituents as such of one mouse, if inoculated into another individual belonging to this strain, are not able to call forth immune processes in this mouse and that the immunity demonstrated under these conditions must be due to factors other than constituents which normal tissues and tumors have in common. However actually a fully homozygous condition has not been achieved in any of these closely inbred strains and the genetic constitution of all the different C3H individuals is therefore not identical; and inasmuch as the method used by Gross makes possible the demonstration of very fine degrees of immunity, it might be expected that in a number of mice belonging to this inbred strain an immunity can be shown to exist under the conditions of these experiments. If the interpretation of Gross were correct, it should be possible by similar means to call forth an immunity against an autogenous tumor, in the mouse in which the tumor originated. But even if such an experiment should succeed, which is not very probable, it would not be permissible to conclude on this basis that the individuality differentials cannot function as antigens in such instances, but it would indicate merely that under these experimental conditions constituents of the tumor cells other than the individuality differentials which they have in common with normal tissues, acted as antigens.

We believe that the organismal differentials in tumors and in normal tissues from which they have developed are identical, or at least very similar, and that the transformation of normal tissues into tumors does not depend upon changes in the genes which determine the organismal differentials, but upon conditions which are comparable to changes in organ and tissue differentials, although they are not necessarily identical with these. The differences between various tumors, arising in different individuals and in different organs, depend upon the original differences in the organismal and organ differentials of the individuals and of the tissues in which they develop, and in addition, upon certain changes of a special character, among which the production of specific intrinsic growth factors or the invasion of cells by extrinsic agents or viruses may play a role. Additional alterations may take place during the life of a tumor, and in particular during serial transplantations, such as variations in growth energy or adaptations to the constitution of new hosts. Future investigations must determine more accurately wherein these changes of a chemical nature, which occur during the transformation of

normal into cancerous tissue, consist, and what the chemical factors are which different tumors have in common.

By means of transplantation of tumors a considerable plasticity in certain functions of the cells composing tumors has been revealed; this plasticity of function is superimposed upon and interacts with the constancy of the individuality differentials of these tumors. In the case of normal tissues, adaptive processes are either lacking or they are very much weaker than in tumors, and by comparison with the latter, normal adult tissues appear relatively rigid. Because of the complexity and the relatively great plasticity in the reactions of tumors, the behavior of tumors presents problems of great biological interest, especially in view of the fact that tumors are transformed normal tissues, and that various attributes applying to tumors apply, therefore potentially, also to normal tissues. But these attributes become manifest only when normal cells, undergoing transformation into tumors, have reached the equilibrium of cancer cells.

Part V Organismal and Organ Differentials and the Specificity of Tissue Reactions

Chapter I

The Relative Importance of Substratum and of Morphogenic Substances in the Specificity of Tissue Reactions, and the Relation of These Factors to Organismal Differentials

In preceding chapters we have referred to the relations which exist between organismal and organ differentials, and the role which morphogenic contact substances play in the differentiation of tissues, organs, and in the development of organ differentials. We have also referred to the action of morphogenic substances affecting tissues at a distance from the place of origin of these substances, and to the importance of gene-hormones in the realization of genetic determinations. In this chapter we shall continue this discussion and analyze additional conditions of an analogous kind—some of which are effective also in the adult organism—in which the specificity in structure and function depends upon and also manifests itself in an interaction between hormones and factors inherent in certain tissues. Transplantation of tissues, which act either as carriers of the stimuli or represent the substratum, was used as a method for the analysis of these relationships in a number of investigations. This specificity may, in certain cases, manifest itself also in the differences in the reactions to stimulating or inhibiting factors which are observed when these factors act on the tissues of different individuals, species, orders or classes of animals. We may designate the latter kind of relation as an organismal specificity; and if this organismal specificity is of such a nature that the stimulating or inhibiting factor originating in a certain individual, species, order or class of animals, is more effective when acting on tissues of the same kind of individual, species, order or class, than when acting on tissues of another kind of organisms, then we have to deal with what may be designated as specific organismal adaptation between the stimulating or inhibiting factor and the recipient tissue. We may therefore distinguish three kinds of specificities: (a) simple organ or tissue specificity; (b) organismal specificity; (c) organismal specific adaptation.

The problem may arise as to the seat of the specificity of the reactions in such cases, whether it is the hormones and other distance substances or the organs in which they originate, or the tissues on which they act. What factor determines the differences in the behavior of analogous tissues or substances in different individuals, species, orders or classes, or the differences in the behavior

of different tissues or substances, or even of apparently the same kind of tissues at different localities within the same individual? Do the organismal differentials cause variations in the hormones and the tissues in which they originate or in the recipient tissues? As the following analysis will show, as a rule the specificity in the reactions in these cases seems to reside in the recipient tissues rather than in the morphogenic agents.. This conclusion is in accordance with what is known as to the lack of organismal differentials in hormones in the large majority of cases and their presence in the tissues, in which latter, therefore, the individuality of the organism predominantly resides.

A simple specificity of the first type exists, for instance, in the structure of the different parts of the uterine cervix and in the graded interaction of this organ with two kinds of hormones. We shall return, here, somewhat more fully to this condition, to which we have already referred in a different connection in a preceding chapter.

In the genital tract of the female guinea pig there exists a graded change in structure in the direction from the vagina through the different portions of the cervix to the uterus, and correspondingly, there can be demonstrated experimentally a graded responsiveness of these tissues to the two ovarian hormones, the follicular hormone and the corpus luteum hormone. The gradation in the action of the lutein substance is in an opposite direction to that of the follicular hormone. Thus in the system consisting of vagina, cervix and uterus, the response to the follicular hormone is strongest in the vagina and shows a graded decrease in the various portions of the cervix. It has still a definite effect of its own in the uterus, but one that is different from the effect observed in vagina and cervix. Through increasing the amount of follicular hormone the reaction in the middle portion of the cervix, which normally is much less responsive to this substance than the vagina, can be made more distinct; but the same quantity of hormone exerts, then, a still stronger stimulating effect on the vagina. In general, the greater the amount of hormone which is allowed to act, the greater the proportional response of the various tissues, this response being always relatively greater in the vagina than higher up, and decreasing the more the nearer the tissue is to the uterus. The reverse relation is noted in the case of the lutein hormone. This exerts a very strong effect on the uterus, which extends only to the directly adjoining part of the cervix, while in the vagina and presumably also in the lower portion of the cervix it exerts mainly an antagonistic and inhibiting influence on the follicular hormone, thus favoring a resting condition in these organs, which otherwise would be stimulated by the latter substance. The most interesting feature in this connection is the graded character of these reactions. Apparently we have to deal with a graded difference in the state of sensitization of these tissues, which either leads to the binding of a graded amount of hormone by the various tissues and thus to a gradation of the reactions, or causes a difference in the responsiveness of the tissues after they have combined [illegible] amount of hormone. In this case there is thus a specificity in the [illegible] same organism with two hormones [illegible] structure of these tissues. We

have therefore to deal directly with organ and tissue differential substances and structures, and only indirectly with organismal differentials, the significance of which is indicated by the fact that in certain other species these tissue reactions may slightly differ.

There is another condition of morphogenic character, in which likewise quantitative relations seem to exist between hormones and specific activities of tissues; namely, in the origin of mammary carcinoma of mice. It can be shown that in individual mice and in different strains of mice there exists a quantitatively graded tendency to acquire carcinoma of the mammary gland. It can furthermore be shown that through a quantitatively graded diminution in the activity of the ovarian hormones, which normally set in motion the growth of the mammary gland, the frequency in the development of carcinoma and the intensity of the reaction, as measured by the length of the latent period preceding the appearance of the tumor, can be reduced in a graded manner; or expressed differently, the length of the time during which the hormone must act in order to produce the carcinoma varies in different individual mice and strains of mice and can be altered experimentally. There is some evidence for the conclusion that here, also, quantitative differences in the response of the recipient tissue, namely the mammary gland of different individuals, depend upon different degrees of sensitization of the reacting tissues rather than on differences in the quantities of the hormones acting in different individuals and strains, and that these differences in the responsiveness of the mammary gland tissue determine the relative incidence of mammary gland cancer in mice.

It is likewise by means of hormone action that it has been possible to demonstrate the fact that in different parts of the body, differences exist in the constitution of the same type of recipient tissues, which morphologically seem to be identical, and that therefore a much greater individualization of tissue differentials within the same organism exists than could have been foreseen. It can be shown that the action of the corpus luteum hormone on the connective tissue in the mucosa of the genital tract of the guinea pig is very selective; it is only the connective tissue of the uterus, but not that of the central or of the vaginal portion of the cervix, nor that of the fallopian tube and vagina, which in the guinea pig responds to the stimulation of this hormone with the formation of decidual tissue, and this is true equally of the tissue in situ, as well as of transplanted tissue. The connective tissue of the uterine cervix responds to the lutein hormone, but with a decreased intensity as compared with the response of the uterine mucosa. We may therefore conclude that the chemical structure and function of the ordinary fibrillar connective tissue differ in adjoining and related organs.

Even adjoining parts of the ordinary epidermis of amphibian anuran larvae are differently constituted, as is shown in a graded response to certain hormone-like substances. Thus the skin covering the root of the tail is more resistant to the injurious effects of substances which induce metamorphosis than the skin at the tip of the tail, the former behaving more like the skin of the trunk of the larva; we shall refer again in a later chapter to this difference

in the reactions of epidermal tissues. Another instance of differences in the constitution of an apparently homogeneous tissue has already been noted; it was shown that different areas of skin of amphibian embryos exhibited different degrees of responsiveness to the contact action of the optic disc, some areas possessing, others lacking the ability to form a lens.

In agreement with these conclusions is the observation that fibroblasts obtained from the connective tissue of different areas of the embryo may behave differently when cultivated in vitro (R. C. Parker). They differed in their rapidity of growth, in the amount of acid produced, and in their power of resistance to injurious conditions, and these differences were permanent in certain strains of fibroblasts and seemed to be inherent in the cells. Not only were variations found in these respects between periosteal, perichondral and ordinary connective tissue cells, but even between connective tissue cells taken from the stroma of various organs. Although in these cases we have to deal with lower organisms and with not yet fully differentiated embryonal or larval instead of with adult tissues, it is evident from our findings in the uterus that in principle the same condition holds good also in the case of adult mammalian organisms.

We may then conclude that the *differentiation of tissues is in reality* much furthergoing than has been assumed on purely morphological grounds. Furthermore, the possibility must be considered that the contact substances, and in certain cases perhaps also the hormones, given off by tissues which are morphologically indistinguishable from one another, may correspondingly differ.

More recent studies of various authors prove the still wider applicability of this mode of experimental analysis of the specific character of certain tissues in embryonal development, as well as in adult organisms. By these means Ritter and Blacher have studied the cause of the differences in pigmentation which are observed in two races of urodele amphibia, the black and white Axolotl, and in different areas of the skin of the same individual Axolotl.

The white and black races of Axolotl differ in the proportion of the pigmented and unpigmented parts of their skin; in the former the white, and in the latter the black color predominates. Now, it is known that in the hypophysis there is produced a hormone which causes a black coloration of amphibian skin by inducing the expansion of the chromatophore pigment and also by increasing the number of these pigment cells. The question arose, therefore, as to whether the inherited difference in the behavior of the skin of the white and black Axolotls might be due to inherited differences in the amount of hormone produced by the pituitary glands of these two races, or whether it was due to differences in the recipient skin. Experiments by E. Ritter have shown that the second interpretation is correct, no difference being noticeable between the hypophysis and its pigment-regulating hormone of the black and the white Axolotls. The difference between these two races consists not only in the greater number of pigmented cells in the black as compared with the white race, but also in the reactivity of these two kinds of

skin. If through extirpation of the pituitary gland the number of chromatophores has been diminished, and if subsequently the pituitary hormone is experimentally again introduced into such an animal, either through transplantation of hypophyseal gland tissue or through injection of the active substance, the skin of the black race responds more readily with the new formation of pigment cells than the skin of the white race. But under these conditions injection of hormone or transplantation of hypophyseal tissues does not entirely restore the normal characteristics of the skin, the number of new pigmented spots remaining smaller in the hypophysectomized than in the normal individuals of the black race of Axolotls. The essential point, however, is that there is no noticeable difference between the action of hypophysis of the white and of the black race, both being about equally effective. The distinguishing features in the pigmentation of these two races depend upon conditions inherent in the structure of the skin; after transplantation of skin from the white to the black Axolotls, and vice versa, the transplants retain their race characteristics. Therefore, factors inherent in the substratum on which the hormone acts primarily determine the pigmentation of the skin. On the other hand, if through transplantation of an excess of hypophyseal tissue into an Axolotl belonging to the white race the quantity of hormone action on the substratum is much increased, then also the skin of the white race can be converted into black skin. The conclusion may then be drawn that the threshold of hormone action necessary to call forth production of pigment cells is greater in the white race than in the black race, and that correspondingly more hormone is needed in the skin of the former to obtain the same amount of pigmentation as in the black race. In this case we have to deal with an example of the second type of specificity, the organismal specificity.

In various classes of animals the skin of the same individual may be white in certain areas, while in others it is black; here, also, the coloration depends not upon differences in the activity of the hypophysis but upon differences inherent in the skin; and again, the threshold in the reaction to pituitary hormone differs in the pigment cells in different areas of the skin. Thus Blacher has shown that after extirpation of the hypophysis the pigment contracts first in the chromatophores of the abdominal skin, next in the chromatophores of the tail and dorsal skin, and lastly, in the corresponding cells in the skin of the head. As a result of the contraction a whitening of the skin takes place. Corresponding to this difference in the reactivity of the chromatophores is the greater tendency of the skin of the head to be black, than of the skin elsewhere; the same difference between the different areas of the skin is found in the black as well as in the white races; also in the latter the skin of the head has the greatest tendency to assume a black color under the influence of the hypophyseal hormone. While thus the differences in the behavior of pigment cells in different areas of the body are of the same type in the white and black races, the threshold of hormone action necessary to cause expansion of the pigment and call forth a newformation of chromatophores differs in the two races.

Blacher and Ritter assume that the differences in the reaction of these cells

depend upon different threshold reactions of the pigment cells to the hormone, the amount of hormone needed in order to obtain an effect being different in the pigment cells in different races, as well as in different areas within the same individual. There remains, however, the possibility that different cells may vary primarily in their ability to attract and to bind a certain amount of the hormone, rather than in the amounts necessary to call forth a reaction. As to the causes of the differences in the behavior of these different types of cells, nothing definite is known, but it may be suggested that a substance is produced within the cell which increases the sensitiveness of the latter to the hormone, a condition analogous to the sensitization to mechanical stimuli which is produced in the uterine mucosa by the lutein hormone.

We see, then, that the same mechanism applies to a condition of pure organ- or tissue-specificity, and to a condition of combined organismal- and tissue-specificity.

Analogous are certain findings in adult mammals. Here in women past the menopause the ovary no longer reacts to the stimulating action of pituitary gonadotropic hormones with maturation of follicles and corpus luteum formation, although the human anterior hypophysis is still potent (Saxton and Loeb); the lack of ovarian responsiveness must, in such instances, be due to changes which have taken place in the recipient organ, the ovary.

In mammals differences in the reaction of analogous organs to the same kind of hormones have been observed in different species. Thus, for instance, the ovary of the guinea pig, rat and rabbit react quite differently to the same gonadotropic hormones of the pituitary gland and to changes in the constitution of hormones which follow hysterectomy. These differences depend on the structure of the ovaries in these species and in particular on the power of resistance of follicles and corpora lutea to injurious conditions and on the ability of the theca interna to undergo luteinization. In these instances we have to deal with organismal specificities in the reaction of tissues to the same kind of hormones.

A similar problem as to the relative significance of substratum and stimulus in determining the specificity of the reaction arises in the field of regeneration. Triton is able to regenerate tail as well as anterior and posterior extremities; anuran amphibia, such as toads, do not possess this regenerative power. In the lizard the condition is intermediate; the tail is able to regenerate, while the posterior extremities regenerate only in a rudimentary way, and the anterior extremities not at all. Weiss transplanted in Triton the regenerative bud from a tail to a cut surface in the anterior extremity, a piece of which had previously been excised. It seemed that the grafted tail material became transformed into a leg under the influence of the leg stump, which thus acted as an organizer and caused the transformation of potential tail material into a limb. In this case evidently the stimulating tissue and not the recipient tissue determined the fate of the tail bud. However, when a similar experiment was carried out in the lizard, where the tail still has the power to regenerate but the anterior extremity lacks it, the transplanted tail bud was not transformed into a leg, because the wound surface of the limb to which it was attached

was not able to restitute lost parts. Weiss concludes therefore that the leg has lost the ability to act as an organizer. However, the interpretation of these experiments suffers from the difficulty that there is some uncertainty as to whether, in Triton, a real transformation of the grafted tail into leg took place, or whether, instead, a regeneration may not have proceeded from the remaining stump of the limb.

On the other hand, Guyénot had shown previously that if, following metamorphosis, an extremity of Bufo vulgaris, which no longer possesses the ability to regenerate lost parts, is grafted to a larva of Salamandra maculosa, which latter is able to regenerate extremities, the transplant heals in but has not gained thereby the power to regenerate lost parts when a portion of the transplanted limb is amputated. This indicates that the lack of regeneration depends upon conditions inherent in the transplanted Bufo tissue, and that the presence in Salamander of substances able to stimulate the growth of a leg, if such substances should exist, is of no avail. In these instances we have to deal with examples of organ rather than of organismal specificity.

Axolotl does not possess a balancer, while Triton does have this organ. But notwithstanding the lack of a balancer in Axolotl, the medullary plate of this species contains an inductor substance able to cause the formation of this structure in the kind of tissue which has the potentiality to produce this organ. Therefore, if the anterior portion of the medullary plate is transplanted from Axolotl to a later gastrula, or to an early neurula stage of Triton, the transplant may induce in the host epidermis the formation of a balancer, while this effect is lacking if the medullary plate is in contact with the Amblystoma epidermis. The reason then why Amblystoma does not possess a balancer is not due to the lack of the proper stimulus, but to the inability of the tissue to respond to such a stimulus in an adequate manner.

The analogy between this condition and the findings of Schotté, to which we have referred in a preceding chapter, is evident. After transplantation of Rana tissue to Triton the oral region of the host supplied an organizer substance, which induced the formation of mouth organs in the transplant; but the potentiality of the transplanted tissue itself determined the specific kind of mouth organs which actually developed under the influence of the inductor tissue. In both of these cases we have to deal with examples of organismal specificities inherent in the reacting tissues, whereas the organizer substance does not manifest an organismal specificity.

There exist, however, conditions in which the lack of reaction is due not to the specificity of the recipient tissue but to the lack of a hormone. Thus Wigglesworth, Piepho, and others, have demonstrated that in the larvae of insects changes leading to pupation are induced by a hormone which is localized in certain parts of the brain—or rather in the ring gland situated between the hemispheres of the larval brain (Hadorn and Bodenstein)—and which may circulate also in the bodyfluids. Now, it can be shown that changes characteristic of the pupa may be induced even in the skin of the imago if the larval pupation hormone is supplied. This hormone does not possess finer organismal differentials and presumably lacks them altogether; therefore it

may be active also in distantly related species of insects. However, while in this case the skin of the distantly related imago, if properly stimulated, still possesses the ability to produce a cuticula, which ordinarily is produced only by the larva, the kind of changes which take place in the skin under the influence of this hormone, the structure and pigmentation of the newly formed cuticula, possess the characteristics of the imago skin. The modifiability of this tissue under the influence of a specific hormone, obtained from a distant species, is therefore restricted. However, if the skin of the imago undergoes regeneration, its potentiality to react like larval skin is restored to it and now the typical changes in the cuticula may be produced by the hormone.

Similarly, Piepho has shown that a larval hormone may induce the normal skin of a pupa to form the cuticula characteristic of the pupa, while regenerating skin regains the ability to produce larval cuticula. In the latter instance the initiation of growth processes in the skin enlarges the range of reactivity of this tissue to specific hormones; when it has reached a more advanced stage of regeneration it behaves like tissues of earlier embryonal stages, which are as yet less differentiated; it returns to a more plastic condition in which the equilibrium is more labile and in which certain changes in the inner or outer environment may cause fargoing transformations. But it seems that the effects of regeneration in increasing the range of reactivity of tissues decreases with increasing phylogenetic evolution, being much less in mammals than in invertebrates. We have seen that the very plastic material of phylogenetically primitive organisms, such as planarians, reacts readily to environmental changes with modifications of organs, whereas the reestablishment of the original set of environmental conditions may lead again to the restoration of the original tissue structures and tissue equilibrium, as the recent experiments of Child have shown. In the very primitive and very plastic material of certain coelenterates the tissue equilibrium may be determined by a set of relatively simple conditions in which mechanical factors and oxygen supply (Barth) may play a significant role.

Also, in the early ontogenetic stages the as yet less differentiated tissue may react to stimulation by specific hormones with tissue changes which correspond more to the specificity of the hormone than of the tissue. Thus in sufficiently early embryonal stages of birds (Willier) and mammals (Ivy) male and female sex hormones can determine in which direction, female or male, the sex glands of the embryo shalt develop.

In our experiments on the production of maternal placenta and placentoma in the uterus of the guinea pig, we analyzed by means of transplantation of pieces of uterus, the interaction between certain morphogenic distance substances and organismat differentials. We found that the formation of placentomata depended upon the amount of lutein substance which has had a chance to act on the uterus previous to, as well as following transplantation, and action at both these periods was necessary in order to obtain the development of large-sized placentomata. There entered into these reactions, furthermore, a mechanical, stimulating factor, which was introduced during the process of transplantation. But in addition the effect depended also upon

the organismal differentials of host and transplant, the transplant showing a marked sensitiveness to the injurious action of homoiotoxins. In this case the morphogenic substance, the lutein hormone, does not bear an organismal differential and the injurious action of the homoiotoxin is due to the sensitiveness of the tissue on which the hormone acts.

Somewhat related conditions were found in the compensatory hypertrophy of the thyroid gland, a process which in all probability is caused by a change in the normal balance between the thyroid-stimulating hormone of the anterior hypophysis and thyroxin, the hormone of the thyroid gland, the former inducing, the latter inhibiting hypertrophy. If we diminish the quantity of the thyroid hormone by extirpating a part of the gland which produces it, hypertrophy takes place; if we increase the quantity of thyroid hormone, hypertrophy is prevented. Although these hormones do not carry homoiodifferentials, still, homoiotransplantation of thyroid tissue interferes with the development of hypertrophy, because the homoiotoxins have an unfavorable effect on the graft.

Similar problems arise in the analysis of the factors underlying metamorphosis. How far do the conditions initiating metamorphosis reside in the tissues and depend upon the organ and organismal differentials of the latter, and how far are they due to the action of stimulating or regulating substances circulating in the bodyfluids and comparable to hormones? It is again largely by means of transplantation experiments that the analysis of metamorphosis has been carried out. In his early experiments of joining together parts of frog larvae, Born had observed that the two partial larvae, when they were combined, metamorphosed at the same time, irrespective of the state of nourishment of the two partners; this may be taken as an indication that one partner influenced the time of metamorphosis of the other. Such an influence was also noticeable in the more recent experiments of Burns, who accomplished a union between larvae of Amblystoma tigrinum, which normally metamorphose very slowly, and those of Amblystoma punctatum, which metamorphose more rapidly; under these conditions Amblystoma punctatum caused a definite acceleration of the metamorphosis of the tigrinum larva.

A furthergoing analysis of the factors underlying metamorphosis has been accomplished through transplantation of pieces of amphibian skin and of the iris of the eye, in which, normally, characteristic color changes take place during metamorphosis. In this way it has been possible, within certain limits, to determine how far organismal differentials influence these processes, and in particular, whether an interaction takes place between the factors determining metamorphosis and the homoio- and heterotoxins which may act on the grafted tissues. From the older experiments of Uhlenhuth on the eye, of Weigl on skin, of Kornfeld on the gills of urodeles, and from the more recent experiments of Lindemann on the skin of frog larvae, we may, in general, draw the conclusion that a chemical factor, a substance circulating in the bodyfluids of an amphibian, initiates metamorphosis synchronously in all the tissues which are sensitive to the effect of such a substance and which are subject to metamorphosis. Furthermore, this substance is able to act not

only on the tissues of the same individual, but also on tissues transplanted from another individual of the same, or even of a different species; if it is present at the time just preceding transplantation in a larger quantity in the host than in the donor, the metamorphosis of the transplant tends to be accelerated; but if present in a larger quantity in the donor than in the host, then a relative retardation in the metamorphosis of the transplant, as compared with the metamorphosis which would have taken place in the donor, is apt to occur.

However, in addition to these factors, others which are present in the transplant influence the character and time of metamorphosis. Among these latter, primary factors inherent in the structure of the tissues, and secondary ones depending on variable environmental conditions, can be distinguished. Thus, the iris of the eye in salamander and also the gills in urodeles undergo certain changes apparently under the influence of specific substances, which become potent some time previous to the onset of metamorphosis, but the mode of action is influenced by specific characteristics of the tissues. Even skin from different surface areas of the same animal may differ as to its reactivity to these substances. According to Lindemann, the skin of the tail of frog larvae will undergo absorption during metamorphosis, and this takes place irrespective of whether the skin has been left in its normal place or whether it has been transplanted into other parts of the body surface. On the other hand, dorsal skin will remain unchanged, even if transplanted into a place which undergoes retrogressive changes during metamorphosis. The condition of the tissues of the donor may modify the metamorphosis in still another way: if the donor organism at the time of transplantation has reached a stage nearer to metamorphosis than the host, the transplant has a tendency to metamorphose at an earlier date than the host; if, on the contrary, the donor is still farther removed from the stage of metamorphosis, the transplant tends to require a longer time before metamorphosis can take place. It seems therefore that preceding the processes occurring during metamorphosis there are preliminary changes in the tissues, which make the latter more responsive and gradually sensitize it to the substances causing metamorphosis, and this process of sensitization requires a certain time. It is possible that the sensitizing substance is identical with the metamorphosis-inducing substance. We may then assume that this substance gradually accumulates in the organism, combines with the responsive tissues and thereby makes them ready for metamorphosis, which takes place after a certain point of tissue saturation has been reached and after the hormone has had a chance to act on the tissues for a sufficient length of time. The possibility also exists that the sensitizing substance differs from the metamorphosing substance and merely makes the tissues receptive to the action of the latter substance. However that may be, a tissue thus sufficiently prepared undergoes metamorphosis after transplantation, even without the presence of the active metamorphosing substance in the host, whereas a tissue not fully prepared or sensitized is not sufficiently responsive even if the metamorphosing substance of the host is fully active. Such a transplant will, therefore, not metamorphose synchronously with the

host organism, but at an earlier or later date, in accordance with its sufficient or insufficient sensitization. Yet, within a certain range of sensitization the hormone active in the host at the time of metamorphosis tends to induce metamorphosis in the transplant synchronously with that of the host tissues.

As to the significance of organismal differentials in the process of metamorphosis, in urodele amphibia the effects described can be noted after auto- and homoio-, as well as after heterotransplantation of the respective tissues, but in anuran amphibia W. Schultz observed that only auto-, but not homoiotransplanted skin takes part in metamorphosis. With the progress in phylogenetic development, the tissues become more and more specialized and the organismal differentials more selective, so that only if the latter are nearly related in the host and the transplant is the metabolism of the transplanted tissue such that the graft is able to respond effectively to the metamorphosing substances.

In the experiments cited in this chapter certain tissue reactions may depend upon the interaction between several sets of factors: namely, (a) conditions inherent in the tissues and determining their ability to undergo certain changes; (b) the action of hormone-like distance substances circulating in the bodyfluids of the host, combining with the transplanted tissues and thus causing their specific effects; (c) the time when these hormone-like substances act on the tissues. In some cases substances of this kind must act not only following but also previous to transplantation, and thus sensitize the tissues; (d) the action of organismal differentials affecting, as a rule, the transplanted tissues and, much less or not at all, the hormone-like substances, although in some cases hormones may possess some of the less specific organismal differentials; (e) mechanical factors acting as stimuli in combination with sensitizing agents; as in the formation of maternal placentomata; (f) a balancing action between conditions inherent in the tissues and the effects of the hormone-like distance substances.

A balancing action, similar to the last mentioned factor, may take place in ordinary transplantation of tissues. In this case we have in all probability to deal with an antagonism between factors inherent in the transplant and contact substances residing in the tissues of the host which serve as a soil for the transplant. Thus in the experiments of Milojevitch, with transplantation of regenerative buds of extremities, the surrounding host tissues apparently determined what kind of limb was to develop, their influence dominating over the conditions inherent in the transplants; on the contrary, in the experiments of Ruud, with transplantation of embryonal limb buds, factors inherent in the transplants determined the result.

In this connection we may also refer to Goldschmidt's theory of the mechanisms underlying Mendelian heredity, and in particular to his interpretation of the sex-intergrades which result from hybridization of different races of Lymantria. In the various types of hybridizations there takes place a mutual interaction of germ cells possessing different genetic constitutions and therefore also different precursors of organismal differentials. Goldschmidt attributes different potencies to various allelomorph genes or gene combina-

tions in the fertilized ovum. The greater the potency, the greater and the more rapidly reached will be that amount of a hormone-like substance sufficient to direct the development of the egg into certain channels. The earlier the threshold is reached at which this substance becomes active, the earlier and more extensive will be its influence on the embryonal development and the more fundamental will be the changes produced, while a substance developing late and in small quantities will affect only the latest phases of embryonal development and its action therefore will be less far reaching. Hence, the effect of such a substance depends upon its potency, quantity, and time of appearance, and also upon the character of the substratum on which it acts and on the intensity and rapidity of the processes which it influences. In a preceding chapter it has been pointed out that the time-factor plays a role also in the interaction between organizer and recipient tissues and that these time-relations may differ in the case of homoiogenous and heterogenous tissues. In heterotransplantation, therefore, incompatibilities may develop between the action of the organizer contained in the transplant and in the recipient tissues in the host, or vice versa. There is, here, an additional interesting analogy between the processes of fertilization and transplantation.

In a somewhat similar manner, according to F. R. Lillie, the gynandromorphism which is occasionally found in birds depends upon certain quantitative variations in the interaction between factors residing in the tissues and the hormone acting upon the latter. In gynandromorphic individuals one side of the animal has male and the other side female plumage, and at the same time the quantity of the female sex hormone which is produced by the ovary is diminished. Lillie assumes that the female sex hormone, under these conditions, is able to impress upon the feathers the female characteristics only if the growth rate of the feathers during their development is sufficiently slow to allow the female sex hormone to become effective, otherwise the feathers assume the male characteristics. Lillie observed also that the side on which the male feathers developed in some birds was often hypertrophic, and he assumes therefore that the rate of growth was too rapid on this side to give the female sex hormone a chance to endow these feathers with female characteristics. Thus they remained male on the hypertrophied side, since here the threshold of reaction for the female sex hormone would need to be higher on account of the more rapid tissue growth. In this case there would be an interaction between intrinsic and external factors, the latter being represented by hormones which, in combination with the inherent properties of the recipient tissue, determine the character of the developing plumage.

Cell and tissue differentiation and loss of differentiation (dedifferentiation), as well as metaplasia, present the problem as to how far factors inherent in the tissues and how far environmental factors, including the inner environment, and, in particular, contact substances and hormones, play a role in these processes. There is a strong indication that as a rule both intrinsic and environmental factors are active, but in varying proportions in different areas, and that with advancing development the intrinsic factors of the

substratum begin to predominate more and more over the environmental stimulating factors, although some general conditions, such as the action of more specific hormone-like substances and the state of nourishment and oxygen supply, remain of importance. In regard to the latter factors, the degree of differentiation may depend, in some instances, upon the influence which relatively unfavorable conditions of nourishment exert on the tissues. To cite an example: there can be little doubt that the keratinization of the epidermis is due to the distance of epidermal cells, undergoing this change, from the capillaries, a distance which increases in proportion to the intensity of proliferation of these cells. The same factors may also affect the rapidity and character of cell division; a certain degree of unfavorableness in a constellation of factors may act as a stimulus, initiating cell division, but at the same time the unfavorable conditions may make the cell division thus induced irregular. On the other hand, conditions which induce rapid normal cell division may thereby inhibit a complete differentiation of the resulting tissues.

Experimental analysis of tissues by the use of hormones, as well as by other means, has made it possible to establish the existence of a much greater differentiation and individualization of tissues in different areas than had previously been assumed to exist, when observation of the structure of tissues seemed to indicate their identity. Furthermore, the characteristics of tissues and organs of an organism are determined by factors inherent in the recipient tissues as well as by stimuli acting on them. While in different cases the relative importance of these two sets of factors varies, in many instances the former preponderate. This is true at least when the more differentiated, phylogenetically and ontogenetically mature stages have been reached, while in the more primitive stages the stimulating and transforming effects of contact and distance hormone-like substances play a prominent role.

There must be added to these morphogenic substances, certain vitamins which also can be shown to have specific morphogenic effects under some conditions; this holds good, for instance, in the case of vitamin A, the absence of which may produce a transformation of cylindrical into squamous epithelium in some epithelial membranes; also of vitamin D, which affects in a specific manner the bony structures. With furthergoing phylogenetic and ontogenetic development certain tissue differentiations take place, requiring the presence of definite vitamins for the maintenance of normal structure and function.

The problem which we have discussed in this chapter is a part of the wider problem as to the relative significance of living substratum and environment in the development and function of living matter, whether of individuals, species, or wider classes of organisms. The tissues which are the bearers of the organismal and the organ and tissue differentials and their precursors represent the substratum, and in this substratum the organismal differentials and their precursors are the most constant constituents, while the organ differentials seem to be more modifiable; the contact substances and hormones represent a part of the inner environment, which however, can

be experimentally introduced also from the outside. In all the cases discussed we notice the relative preponderance of the character of the substratum over the environmental factors. Nevertheless, in the course of investigation, it has been found in many cases that what had hitherto been assumed to be determined solely by conditions inherent in the substratum, is determined in part also by environmental factors, the latter thus growing in importance. The further extension of the conscious control of life processes depends on the discovery of additional extrinsic factors influencing tissue reactions, and the possibility of modifying these experimentally.

Chapter 2

Structure and Function of Organs and Tissues as Criteria of Individuality

OUR RECOGNITION of and distinction between different human individuals depends on many factors, particularly on their facial features, the color of hair and eyes, the height and outlines of their bodies, the character of their movements, especially their way of walking, the quality of their voices and modes of speech, thinking and feeling, in general on their reactions under varying conditions. By these means we can distinguish between individuals and we conclude that no two persons whom we meet are exactly alike; this holds good even of identical twins. But a certain experience is necessary in the use of these different signs. We can best differentiate individuals who, in the most essential features, are similar to those we meet daily in the greatest number, and we have more difficulty in differentiating between classes of individuals with which we are less well acquainted; for instance, it is more difficult for most of us to distinguish between individual monkeys and dogs, than between human beings, although persons who are studying monkeys and dogs very closely can, here, also quite readily distinguish different individuals. We use the combination of a large number of organ and tissue peculiarities and the general body build as distinguishing marks between individuals, each individual thus representing to us a mosaic, which, as a rule, leaves in us a composite impression rather than a memory of the separate elements constituting the mosaic. These separate features are determined largely by inheritance, although variable environmental factors may greatly influence their ultimate character, and different kinds of characteristics are unequally affected by the genetic constitution of the individual and by environmental factors, the experiences through which the individual has passed.

We shall discuss here, in particular, two characteristic features which distinguish human individuals and which are especially striking as to their fineness of individualization, namely, the skin patterns, which are employed for the identification of individuals, and the scents which dogs use in tracing the movements of and in recognizing individuals.

1. *The palmar and plantar skin patterns as criteria of individuality.* The patterns due to the arrangements of the ridges in the skin of the palms of the hand and the plantar surfaces of the foot differ, but are constant in each individual; in the form of finger prints they are used to distinguish individuals from one another. These peculiarities are distinct from the individuality differential, inasmuch as they are limited to one particular organ and do not represent a characteristie shared by all or the majority of the organs or tissues of the body. In this respect they resemble therefore other

individual peculiarities, such as the color of hair and of iris, the cephalic index, or similar characteristics, which, all combined, represent the mosaic structure of the organism, in contrast to the essential individuality, which is based on the organismal differentials. However, while many persons have brown hair or blue eyes, the pattern of the skin ridges, as stated, is specific in every individual. The ridge patterns of the skin, together with other mosaic characters, differ as to the frequency with which certain peculiarities are present in different races, the difference between races being, therefore, largely statistical, the same structural elements as a rule occurring in all of them. The papillary skin patterns have this statistical characteristic in common also with the factors on which blood grouping depends, certain blood groups being found more often in some races than in others. But, like the individuality differentials, the skin patterns represent individual characteristics, while the features distinguishing the four original blood groups do not, to the same degree, allow the differentiation of individuals; they represent group characters, as their name implies.

In the case of monkeys, the parts of the skin where these ridges are found serve as instruments with which to hold fast to trunks of trees and other objects of a similar character, and those types of ridges which may be of use in this function have been designated as "friction ridges." According to the description given by Wilder and Wentworth, the ridges run, in general, in an approximately parallel direction over the greater part of the friction skin, more or less across the longitudinal axes of hand and foot, but in certain definite places where the surface rises slightly, to come into fuller contact with external objects, there occur some departures from the usual course and the ridges form loops, typical concentric whorls and spiral whorls or spirals. These patterns are arranged about a central core which corresponds to the center or summit of the mound. At the point of origin of certain ridges of the patterns, formations may be found which resemble the letter delta and are therefore called "deltas." In addition there are distinguished some ridges which connect the bases of adjoining whorls, and according to the mode in which these ridges end, they are designated as inner and outer melting whorls. Furthermore, there can be recognized the socalled "details" first described by Galton: the forks, ends, islands and enclosures, signifying the length and shape of certain interrupted portions of ridges.

Pairs of *symmetric fingers* in both hands may have their own peculiarities. In one finger pair one pattern may predominate, and in another pair, another pattern. The number of ridges may differ in certain areas of the skin in different individuals, and this quantitative factor behaves in a corresponding manner in each finger pair in the same individual. Taking all these peculiarities together, it is impossible to duplicate the character even of a relatively small area of friction skin in two individuals. The ridges are a permanent bodily mark, never changing throughout life; they originate in the embryo and even after injury they form again in the same manner, unless the injury has led to the destruction of the entire epidermis over a given area.

As stated above, the average frequency with which certain patterns occur

differs in individuals belonging to different races. However, the relative frequency of these patterns in different finger pairs is similar in all races. The ramifications of the cutaneous nerves seem to determine the situation of the cores of the patterns and the distribution of the nerves apparently determines also the number and frequency of ridges in a unit area. In addition, the differences in the shape of embryonal fingers, and especially in their surface radius, influence the character of the patterns. These determining factors underlying the formation of the patterns, which are thus complex in nature and, to some extent, separate and independent of one another, are largely transmitted hereditarily from parents to offspring in the same way as other characteristics constituting the bodily and psychical mosaic.

Of special interest, therefore, is the study of the skin patterns in so-called identical twins, and here it has been found that the number of ridges composing a pattern are much more similar than in bi-oval twins. But even in identical twins certain variations in the patterns develop. It is therefore assumed that these variations are phenotypic in nature, that is, they are partly determined by environmental factors which are different in each twin, and these latter variations are superimposed upon the genetic factors, which are identical in both.

There exist, also, sex differences in the skin patterns, but these disappear in old age with the cessation of sexual function; they may therefore be considered as constituting secondary or tertiary sex characters.

The correlation, noted by Poll in human beings, between skin patterns and certain characteristics of parts of the nervous system, especially conditions which lead to insanity, is of interest. This investigator finds that certain patterns predominate more in normal, and other types in insane persons, but only in the male. However, as in the case of race characteristics, we have here also to deal merely with statistical differences, the frequency with which certain characteristics of skin ridges occur differing in normal and in certain insane persons. Poll holds that this correlation is due to the fact that both the nervous system and the skin are of ectodermal origin, an interpretation not borne out by the findings of Kretschmer, that correlations exist also between the character of the structure of the osseous system and of the panniculus adiposus on the one hand, and the tendency to the development of certain temperaments and of certain types of insanity on the other hand. In a similar way, Graves has observed a certain correlation between the shape of the scapula in a man and his power of resistance to injurious conditions. It may therefore be assumed that the total skin pattern, as well as its single features, depends upon genetic factors in the same way as the structures and function of other systems depend upon genetic factors, and there exist, probably, on this basis correlations between various organ systems, irrespective of their embryonal relationship.

II. *Scents as criteria of individuality.* Distinctive scents emanating from animal organisms originate in the metabolic changes in certain organs; they may therefore be classed among organ characteristics of the kind with which we have to deal in this chapter.

It is well known that the reactions of many animals towards other animals are determined mainly by the sense of smell, which is very much more finely developed in them than in man. This sense of smell plays evidently a great role in the social-psychical relationships of certain insects. It is also apparently by means of individual or family scents attaching to their young that certain animals, for instance, a guinea pig mother, can distinguish their own children from the children of others, and it is this factor which determines the difference in their reactions towards their own offspring. In human beings, this faculty is lost; mothers no longer possess the ability to distinguish babies from each other by the sense of smell. That human beings, too, possess characteristic scents, however, is shown by the fact that dogs can thus readily distinguish different individuals.

As early as in 1879, Gustav Jaeger drew attention to the distinctive scents differentiating human beings as well as human races. He maintained, furthermore, that different species, genera and classes of animals, each have their own characteristic scents, different from those of other groups of animals. As to the origin of scents, some of his conclusions were erroneous. He believed, for instance, that the substances responsible for specific smells were preformed already in the germplasm; similarly, he assumed that the substances, on which the specific sense of taste depends in various species of animals, are present in their germplasm, and that these substances, together with certain pigments which distinguish different races and species, represent the specific constitution of the germplasm. However, it is not these substances, themselves, which are preformed in the germplasm, but rather certain other substances which, in the course of embryonal development, make possible the formation of organs, whose metabolism is of such a nature that the specific scents, tastes and pigments are produced. While, thus, the character of specific scents is ultimately determined by the constitution of the germ cells, the scents as such, represent derivatives of germ cell constituents. Jaeger erred in still another direction. He did not differentiate between the inherited individual or racial scents and others which are due to accidental, social conditions. Traditional suggestions leading to emotional attitudes, the result of certain phases in the social struggle, obscured, in this respect, his judgment.

Subsequently, Correns drew attention to the importance of individual differences in the scent of human beings, but it is especially Löhner who, more recently, has analyzed experimentally the character of individual scents and the reaction of dogs to them. According to Löhner, in human beings there are regional smells distinctive of certain areas of the body, which are mainly seated in the skin and which originate especially in the secretions given off by the sebaceous glands. The different regional smells in the same individual differ very much from one another, from a quantitative as well as from a qualitative point of view, and these differences may be so pronounced that even the human olfactory organ can differentiate them in the same individual. On the other hand, a human being cannot recognize the scent of an individual as a whole, while dogs, especially police dogs, can do so very readily. According to Löhner, such dogs, in addition, are able to recognize even individual

differences in the corresponding regional smells, although it is not certain from Löhner's report that this fact has been experimentally established. However, if this view should be correct, then it would follow that the scent of an individual is not only a composite effect of his multiple regional scents, but there is, besides, a specific feature attached to each regional scent of a given individual. It is of interest also that, secondarily, these scents are influenced by the functioning of the sex organs and that they become quantitatively more pronounced at the time of puberty. It has been found, moreover, that likewise the distribution of the openings of the sweat glands are individual characteristics in man.

We see, then, that in the case of individual scents, as in the case of skin patterns, we have to deal with complex effects which represent the result of the composite actions of more elementary units. Organismal differentials are not involved in either instance, but rather special substances or structures inherent in certain tissues or organs; these localized characteristics are not inherent equally in all, or even almost all, the tissues of an individual, but they are specific for each individual. They must therefore be included among the mosaic characters which distinguish individuals.

III. We have discussed more in detail two inherited conditions in man as examples of individual differences of organs, or tissues, their structures and chemical characteristics. But similar differences are found also between the other analogous organs and tissues in different individuals and species. On the other hand, analogous organs have essential features as to metabolism and function in common in different species, especially in more nearly related ones; the differences which they show become individualized the more, the further advanced these species are in the phylogenetic and ontogenetic scale.

In mice it can be demonstrated that in different, closely inbred strains, various organs such as thyroid and corpus luteum, differ in their structural and, therefore, also functional age curve; likewise, notwithstanding the essential similarity in structure of vagina and uterus, the structure of the maternal placenta in nearly related species shows some notable differences. Furthermore, different species of fresh-water fishes present characteristic differences in their reaction to differences in CO_2 pressure in the water in which they live (Irving). The structure and physiologic reactions of the red corpuscles, the crystalline forms of hemoglobin differ in different species, and these differences are, to a certain extent, correlated with the phylogenetic relationship of the species from which they are derived (Reichert and Brown).

As a further example of this type of specificity, we might mention the manner in which various species or classes of animals react against phenylacetic acid when it is introduced into their bodies. In the majority of mammals, including monkeys, this substance combines with the aminoacetic acid (glycin), and it leaves the body in the form of phenaceturic acid. In man and in anthropoid ape (chimpanzee) phenylacetic acid combines with glutamine, the amide of glutamic acid, a dicarboxy acid, to form phenylacetyl

glutamine. In birds, and presumably also in reptiles, it combines with diaminovaleric acid (ornithin), the principal endproduct of protein metabolism in these classes of animals. Or, to mention another example: while in man and mammals in general, in amphibia and fish the principal endproduct of amino acid metabolism is urea, in birds and reptiles the principal endproduct of protein breakdown is uric acid.

Individual differences in the electric potential of the grey matter of the brain, originating presumably in the structure and function of the ganglia cells, are found when electric currents are obtained with electrodes placed on different parts of the skull or brain surface (Hallowell Davis). A similar individualization in electric potentials also exists in different parts of the eye, and the totality of such potential gradients in the adult and in the embryo seems to be characteristic of different species. Similar findings may be obtained presumably in every organ and every tissue, and we may assume that at least in the higher organisms it might be possible not only to discover species and strain differences in all the organs and their functions, but also individual differences, in accordance with inherited constitutional characteristics, if only we had fine enough methods to recognize them.

In a general way, such organ and tissue differences parallel, in their development, the phylogenetic evolution of these species, but there are many exceptions to this rule. Two examples in which a strict parallelism does not exist may be mentioned, namely (1) the substances which control the expansion and contraction of the melanophores of the skin, become effective in some classes of animals mainly through the nervous system, when they function as neurohormones; in other classes, through the blood, when they function as ordinary hormones. Sex determination depends, in part, on the distribution of two chromosomes in male and female in two possible ways; in vertebrates as well as in insects, these two modes of distribution are found irregularly present, without reference to phylogenetic relationship; likewise the means of control of the state of contraction of the chromatophores are irregularly distributed.

The organism consists therefore of a mosaic of organs and tissues; but the units in this mosaic are subdivided again into smaller units and thus the mosaic is really much finer than it might appear if only gross methods of differentiation are used. It is by means of a more detailed microscopic examination and a study of the mode of reactions, of different tissues to various hormones that very fine subdivisions are revealed, as for instance, in vagina, cervix and uterus of the guinea pig; and this is true of connective tissue as well of epithelial structures. As mentioned already, in tissue cultures of various embryonal structures R. C. Parker has shown that fibroblasts derived from different organs behave differently in regard to rapidity of growth, production of acid, as well as solution of fibrin, and these characteristics remain constant in vitro, although, on the whole, they may change with advancing development. The existence of definite units constituting the organism is also indicated by the study of inheritance of organ characteristics in accordance with Mendelian principles. Furthermore, those factors whose development is controlled by gene-hormones in various insects represent mosaic character-

istics, such as the color of the eye. However, structural and functional subdivisions in the living adult organism do not need to be sharply separated, but transitional areas may gradually lead from one unit to the adjoining one. And all these organ and tissue units, which make up the mosaic of the organism, are connected into one functionally unified whole by means of hormones, including contact substances, and the nervous system.

Accompanying the structure of organs and tissues are their functions. As they are actually studied, they are essentially the functions of species and not of individuals; they are therefore those which are shared by the individuals of a species; they bear the character of species differentials. Of this nature is the tendency to maintain a constant osmotic pressure and fluid content in the bodyfluids, termed by us homoiotonia and homoiohydria, to which might be added homoioproteinemia, the tendency to keep the protein content of the blood constant, and, in general, the condition called by Cannon, homoiostasis, which comprises the sum of all the mechanisms which tend to keep the constitution of the bodyfluids, the milieu interne, within narrow limits constant. However, within these functional mechanisms characteristie of species there are those due to the variations of individuals, of which the species type merely represents the average. In different individuals the functions of different organs may show independent primary variations, which secondarily may lead to adjustments which concern the individual as a whole. These individual differences in organ functions, associated as they are with visible or invisible structural differences, may also be used for the characterization and distinction of individuals.

Besides regulating function, the hormones present in endocrine organs, and similar substances present in other organs, such as bone marrow, liver and kidney, may, in an organ-specific way, regulate also growth, promoting or inhibiting it, and some of these substances may to some extent control the organ in which they originated. But the organs where the hormones are produced, and the various constituents of the nervous system which are endowed with the function of controlling and coordinating other organs, are merely parts of the mosaic organ system. They function by means of their organ characteristics, and the hormones which they produce are not, as a rule, endowed with the organismal differentials which are however present in these organs. But there are indications that some hormones, as for instance, the gonadotropic hormones of the pituitary gland, possess species or class differentials. This specificity applies presumably only to those hormones which chemically have a more complex structure, and which consist of or are combined with proteins. During ontogenetie development, organizers, which at very early stages may induce the reproduction of approximately the whole embryonal organism, but gradually, with the increasing differentiation and specialization of the parts of the organism, become more specialized, exert a controlling, unifying influence in cooperation with the specific substratum on which they act. However, it will be necessary ultimately to trace backward these specific substrata and organizers to simpler structures which represent the precursors of such specific formations.

Combined with the mosaie individuality is the system of organismal dif-

ferentials which help to produce the autogenous or, more generally, the organismal tissue equilibrium, and which are present throughout the various parts of the organism. They represent a second unity and are the foundation of another type of individuality which, in contrast to the mosaic type, might be designated as the essential individuality. From a genetic point of view, tissue and organ characteristics, as well as individuality and species differentials, ultimately depend upon the genetic constitution of individuals and species and there is therefore a close relation between these two factors; however, the number of genes determining them, and perhaps also the character of the individual genes which enter into their composition, differ, and there is therefore no complete parallelism between organ characteristics and organismal differentials.

From the point of view of chemical structure, we may conceive of the organ and tissue units as essentially consisting of a base of proteins which have undergone phylogenetic and ontogenetic development. These proteins may be assumed to be the bearers of characteristics common to all living protoplasm; but on this foundation there are built protein characteristics, first of the largest animal group, to which the organism bearing these organs belongs, and gradually there are added to these in sequence, constellations in the protein, which are characteristic of class, order, family, genus, species, strain and individual. These represent the organismal differentials. There develops also in association with this basic protein structure, phylogenetically and ontogenetically, a subdivision of each organism into a mosaic of organ and tissue units, in which there are added to this protein base, new protein constellations differing in different organs and tissues; or looser associations of the proteins with other, at first presumably very complex, substances of a carbohydrate or lipoid character are acquired. In this case, likewise in sequence, an increasing differentiation of these mosaic organ and tissue units occurs, until in the end the most complex individual is established. Being built upon the foundation of organismal differentials, these units contain the class, order, species and individual characteristics which all parts of the organism have in common; but there develop also in these chemical structures, smaller units which may become detached from the main substance, and which as a rule show less or none of these organismal differentials; these function as enzymes, hormones, and certain other substances. In studying the factors which bind the cellular constituents into the organ and tissue units and which cause the interaction of different organs and tissues within the same individual, specificities characteristic of class, order, species or individual, may be present or may be lacking. In the latter case we can exclude participation of the various organismal differentiats in the reaction or function of these organs and tissues. But if a function or reaction does show such an organismal specificity, then the further question arises as to whether this specificity is to be attributed to the organismal differential chemical groups as such, or to other structurat peculiarities of the organ and tissue units, which presumably originally developed under the influence of the organismal differentials, but which secondarily assumed a constitution

distinct from that of the latter. It may be impossible in many cases to answer such a question. These difficulties arise especially if there are found chemical characteristics in a certain group or tissue of an organism and if these are lacking in other organs. In such a case class or species specificities, which in other instances are due to the existence of organismal differentials, may be due to chemical structures of a different kind in which the organismal differentials are not involved. This question may arise also if we have to deal with characteristics of organs and tissues which distinguish one species, or one individual, from another, but in which these organ differentials do not show the gradations corresponding to the degrees of phylogenetic relationship.

While skin patterns as well as scents are characteristic of individuals and may differentiate one from another, it has not been shown that these structural and biochemical characteristics can be used for determining the relationships between organisms belonging to the same species in the same sense in which individuality differentials can be used for this purpose. This fact does not exclude the possibility that as a result of close inbreeding, continued through successive generations, we might approach a homogeneous population, in which all component individuals would presumably have very nearly the same skin patterns and scents. There are other tissue and cell characters which show a certain group distribution, which is largely independent of individual and species relationship. This is true, for instance, of the agglutinability of the red corpuscles according to which individuals can be assigned to one of the four primary blood groups; although these characteristics may be similar in related species such as men and certain apes. There are other tissue or cell characters, such as the heterophile differentials, which are distributed quite irregularly among different species, without regard to relationship. Some substances show variations in constitution, which, within a definite range, correspond to relationships of species; this seems to be true of the hemoglobins, and there is some reason for assuming that it is true of other kinds of proteins.

However, the larger the number of tissue and organ characteristics of individuals, families and species which we use for identification, the more probably will become the chance that, in their totality, their distribution will correspond to relationships between these individuals, families and species. Thus, if we study various organ systems in different species, a correspondence is found, at least in a general way, between these structures and the phylogenetic relationship of these species; comparative anatomy and biochemistry can help thus in the tracing of phylogenetic relationships, and more intricate studies of the evolution of organ systems may likewise reveal individual and family relationships. We may therefore conclude that various kinds of tissue and organ differentials, whether structural, biochemical, or functional, may serve to distinguish between individuals, and insofar as these characteristics have a genetic basis, they might, in a limited way, even indicate certain relationships between individuals; but they would not therefore become identical with individuality differentials.

Chapter 3

Organismal Differentials and Specific Adaptation of Tissues and Their Products

In the preceding chapters we have used the interactions between whole organisms or parts of organisms, between organisms and tissues or organs, as indicators of organismal relationship; which means the relationship of organisms or of parts of organisms, in accordance with the data of phylogeny; and it is the organismal differentials which express these relationships; but in addition the interaction between certain substances which are produced by organisms, or the interaction of such substances with cells or tissues, may likewise indicate these relationships. If a tissue or such a substance interacts more efficiently with an organ and its products derived from the same phylogenetic group than with an organ or its products derived from a strange phylogenetic group, then these substances or tissues may be designated as "specifically adapted" to each other, especially if the degree of efficiency in this interaction is the greater the nearer the phylogenetic relationship. We have previously discussed various interactions of tissues which are mediated by substances which, as a rule, do not carry the organismal differentials, such as hormones and organizers, the latter functioning as organ- and tissuespecific substances. We also have given some reasons for assuming that certain substances bearing individuality differentials may function as autogenous regulators, which maintain the equilibrium between adjoining tissues; the localized substitution of a homoiogenous for an autogenous tissue may alter the normal activity and relationship of tissues, and there are good reasons for believing that these changes are caused by the character of the substances given off by homoiogenous tissues. However, before we enter into a discussion of such substances, in which organismal differentials determine the specific adaptation of tissues to each other, it might be well to define again the different meanings which may be attached to the terms specificity and specific adaptation, as far as they refer to organisms.

1. The term "specificity" may be applied solely to organs or tissues interacting within an organism, without reference to the organism as a whole; such a condition may be designated as organ, tissue or function specificity. The term "specific" may thus accentuate differences between different organs and tissues within the same kind of organisms. It may include the most important organ and tissue differentials, as well as others of secondary importance; and, furthermore, structural and functional peculiarities which depend presumably on the presence of such differential substances. We have discussed these organ and tissue specificities in the preceding chapter. In a wider sense, this term may also include enzymes and hormones produced by

certain tissues or organs within an organism, because they differ from substances produced by other related tissues in the same organism.

The term "specific" in this sense may refer to a relation between two kinds of substances, or between a substance and an organ or tissue, or between the function and structure of various organs within the same organism, or between an organ or tissue and environmental factors. Such specific relations exist, for instance, between an enzyme and its substratum, between a hormone and the organ on which it acts, between an environmental factor and a particular sense organ, and between various organs and organ systems within the same organism. We have here to deal with intra-organismal tissue, organ and substance adaptations. Specific in this sense are also the relations obtaining in general between parasites and hosts, as well as the relations between man and domesticated animals. These may also depend on tissue or organ specificities but in these adaptations there may participate, secondarily, also the organismal differentials, and these specificities are therefore organismal in character and may be classed with type 2. In general if these organ characteristics are specific of individuals or species they become organismal specificitics, and such organ or tissue specificities characteristic of species are used largely in determining the systematic position of plants and animals.

2. The term "specific" may be used in order to express the fact that a certain structure, substance or function is limited to and characteristic of a certain class or species of organisms, or a certain individual. This is organismal specificity. There is no reference made, in this case to a particular adaptation which this structure, substance or function may bear to others in the same organism. Thus, in certain tropical nymphaeaceae, the pollen-tube grows out and fertilization can occur if the surrounding medium contains a very small amount of boric acid. This is apparently a specific characteristic of these plants and is not known to apply to other plants. In this sense the chitinous integument is specific for certain classes of animals. Limulus and other arthropods have respiratory blood pigments, which are peculiar to these types of organisms. In the metabolism of birds, allantoin plays a specific role. Different hemoglobins are specific for species, and in certain respects, for individuals. We have referred to other similar examples of this kind of specificity in the preceding chapter. While these specific structures, substances or functions may actually enter into relationship with others bearing a corresponding organismal differential, we leave this possibility out of consideration under present conditions. A certain combination of structural, metabolic and functional peculiarities is characteristic of a given individual or species. Also, in the realm of psychical-nervous functions there exist specificities of a similar kind. Thus a certain event calls forth in one individual, but not in another, a peculiar reaction, often depending upon a preceding experience of the first individual, which was peculiar to him and not shared by certain other individuals. While these two types of specificity represent distinct characteristics of organs and tissues, still they appear as a rule associated with each other.

3. The term "specific adaptation" may be used to designate the difference between the results of the interactions of two substances or tissues if they take place on the one hand between individuals A and B, and on the other hand between individuals A and C; and likewise between species S and T and species S and R. These differences in the results of interaction depend upon the character of the organismal differentials of the different individuals or species, and the degree of these differences should then be graded in correspondence with the degree of genetic relationship between these organisms, since the organs or substances involved find, in more nearly related organisms, receptors to which they are better fitted than to those in less nearly related organisms. It is the correspondence between the organismal differentials of organs, tissues or substances in one organism and the receptors of organs, tissues or substances in another organism, which characterizes the specific adaptation in the reaction between them.

Such a specific adaptation can be demonstrated most readily if we have to deal with class and generic differentials. The finer the differentials are which come into play, the more difficult it is to demonstrate a mutual adaptation. Thus, a specific adaptation between substances carrying species differentials can be demonstrated less readily than an adaptation between substances carrying class or generic differentials; and still greater is the difficulty when individuality differentials interact. This increasing difficulty in recognizing the presence of finer organismal differentials may be due to deficiencies in the case of the very finely graded reactions by means of which finer organismal differentials are tested. We would have, therefore, to face in this case the same problem which arose when, in joining together more primitive tissues or organisms, it was possible to demonstrate the presence of the coarser, but not of the finer, organismal differentials.

In all the instances considered so far, we have to deal with the interaction of specifically adapted substances or tissues which are preformed. However, a similar specific adaptation can also arise through active immunization, when one substance serving as antigen, enters the system of an organism belonging to another species; immune substances may then develop, which react with the antigens in a specific and graded manner, corresponding to the relationship of the organisms or tissues and organs involved in these processes. We have here, then, to deal (1) with a specific adaptation between an antigen and an antibody, and in addition (2) with a gradation in specificity in the interaction between antigen and antibody in the sense that other substances may take their place the more readily, the more nearly related organs and tissues, or the organisms are which substitute for the primary antigens or antibodies. Conversely the degree of specific adaptation between these substituted antigens or antibodies may serve as the indicator of the degree of relationship between the primary substance and the substitutes.

In this discussion we have attributed the organismal reactions exhibiting a specific adaptation to the presence of organismal differentials, with which organ-specific substances may be combined in certain cases. However in preceding chapters we have found instances in which specific reactions be-

tween organisms graded in accordance with their relationship depended on substances which were not identical with the primary organismal and individuality differentials. We have encountered reactions of this latter kind for instance in transplantations among embryos which do not possess organismal differentials in the strict sense, but instead possess precursors of these differentials; we have encountered examples of this kind also among unicellular organisms and among algae; but there is reason for assuming that also in other cases the equilibrium between the parts of an organism and its graded interaction with other organisms may depend on substances other than the typical fully developed organismal differentials. We have seen that the characteristics of certain organs and tissues may also be used in the classification of organisms and that the development of the organs and tissues and their differentials from simple structures and substances to more complex, differentiated ones has taken place in association with the corresponding development of the organismal differentials. Substances other than organismal differentials may be involved in the reactions which exhibit specific adaptations between organisms. Some of the substances which are the bearers of these specifically adapted relations seem to be relatively simple, heat resistant substances, of neither a protein nor of a complex carbohydrate or lipoid nature, therefore quite distinct from the organismal differential substances in the striet sense, although the possibility exists that they are derived from the latter type of differentials. Our present limited knowledge does not make it possible, in many instances, to distinguish between these different types of substances and the specific reactions which they cause. We may then apply in these cases the term organismal differentials in a wider sense, which includes substances which are concerned with the production of specific adaptations.

We may now cite some examples of specific adaptations in the interaction of preformed substances which may or may not carry organismal differentials. It can be shown that there exist in the blood sera of various classes or species of vertebrates, substances which in combination with certain other substances, the tissue coagulins present in tissue extracts may cause either an acceleration or an inhibition of blood coagulation, in accordance with the kind of animals from which the sera or extracts were obtained, and in accordance with the length of time during which these two kinds of substances were allowed to act on each other before they were added to the blood plasma which served as test material. Now, there is evidence that the substances in tissue extracts and sera which act together or perhaps combine to form agents accelerating the coagulation of the blood are specifically adapted to each other, and there is likewise a probability that also the inhibiting substances are, in the same sense, specifically adapted to each other. This would mean that the accelerating, and perhaps also the inhibiting, precursor substances in serum and extract carry class or species differentials, and that when substances carrying the same or related differentials interact, the effect on coagulation is greater than when substances carrying disharmonious differentials interact. A specific adaptation is also noticed between tissue extract and blood plasma, the tissue extract of the same class as that from which the plasma has been obtained,

3. The term "specific adaptation" may be used to designate the difference between the results of the interactions of *two* substances or tissues if *they* take place on the one hand between individuals A and B, and on the other hand between individuals A and C; and likewise between species S and T and species S and R. These differences in the results of interaction depend upon the character of the organismal differentials of the different individuals or species, and the degree of these differences should then be graded in correspondence with the degree of genetic relationship between these organisms, since the organs or substances involved find, in more nearly related organisms, receptors to which they are better fitted than to those in less nearly related organisms. It is the correspondence between the organismal differentials of organs, tissues or substances in one organism and the receptors of organs, tissues or substances in another organism, which characterizes the specific adaptation in the reaction between them.

Such a specific adaptation can be demonstrated most readily if we have to deal with class and generic differentials. The finer the differentials are which come into play, the more difficult it is to demonstrate a mutual adaptation. Thus, a specific adaptation between substances carrying species differentials can be demonstrated less readily than an adaptation between substances carrying class or generic differentials; and still greater is the difficulty when individuality differentials interact. This increasing difficulty in recognizing the presence of finer organismal differentials may be due to deficiencies in the case of the very finely graded reactions by means of which finer organismal differentials are tested. We would have, therefore, to face in this case the same problem which arose when, in joining together more primitive tissues or organisms, it was possible to demonstrate the presence of the coarser, but not of the finer, organismal differentials.

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presumably in combination with a factor in the blood plasma, causing coagulation of the fibrinogen more rapidly than that of another class.

A similar type of specific adaptation between substances may influence also the behavior of cells; thus, according to Mudd, Lucké, McCutcheon and Strumia, the macrophages and polymorphonuclear leucocytes of rabbits act as phagocytes towards bacteria, and also towards erythrocytes and protein-coated collodion particles, more efficiently if rabbits sera are used as the carrier of bacteriotropin than when human sera are used. The same specific relation is seen if immune serum is used instead of normal serum. The serum with the tropin which it contains, or the globulin fraction of the immune serum, is supposed to spread over and to attach itself to the surface of the antigenic material. As a result of this effect the spreading-out and phagocytic activity of the leucocytes are stimulated. Under these conditions the leucocytes behave as if they were able to differentiate between the sensitizing substances in the sera of two different mammalian species.

Tillett and Garner, and subsequently Madison and Van Deventer, observed that a filterable, heat-stable substance can be extracted from streptococcus hemolyticus, which fibrinolyzes plasma. Substances from strepocccci isolated from inner human organs dissolve human plasma and, slightly, monkey plasma; cultures of this kind are inactive towards the plasma clots of other animal species, such as the rabbit. In streptococci isolated from horses there is a fibrinolysin that is specific for horse plasma and the same applies to swine streptococci and swine plasma. Addition of serum from the same species, especially of anti-streptococci immune serum, inhibits the action of the fibrinolysin in a specific way. By serial passage of a human streptococcus through rabbits it is possible, according to Reich, to cause in the streptococcus a loss of the human carbohydrate A and a loss of the fibrinolysin for human plasma; instead, a carbohydrate characteristic of animals appears. By subsequent serial cultures of the streptococcus on human blood agar plates, the original characteristerics of this streptococcus are restored.

In a somewhat related way, Duhey finds that the action of serum is specifically adapted to red corpuscles of a certain kind. Thus the venom of Synanceya horrida is hemotoxic as well as neurotoxic. The hemotoxic action against the red blood corpuscles of a given species of animals is much more readily prevented through the addition of serum of the same species than by that of the serum of a different species. Thus rabbit serum protects rabbit corpuscles, while human serum protects human corpuscles; a species differential seems therefore to be attached to an inhibiting substance.

A further analogous condition is noted in the interaction between blood sera and the venom of heloderma; sera which do not activate the hemolytic effect of this venom, inhibit it. Now in some cases hemolysis of the erythrocytes of a certain species seems to be especially inhibited by the blood serum of the same species. Likewise, Besredka observed that sheep serum protects sheep corpuscles, but not the corpuscles of another species, in a specific manner against the hemolytic action of rabbit serum.

In general, the blood serum of an individual is specifically adapted to its

own red corpuscles and the serum of a species is likewise specifically adapted to the red blood corpuscles of this species, although the adaptation between the serum and cells within a certain species is not so perfect as that between serum and cells within the same individual. Therefore autohemolysins do not occur under ordinary conditions and, as a rule, cannot be produced experimentally. Much more common, however, is the appearance of heterolysins. Examples of such a specific adaptation between blood serum and erythrocytes may also be found in invertebrates; amoebocyte tissue which, under certain conditions, results from the agglutination of the amoebocytes of Limulus, remains better preserved in Limulus serum than in the sera of other kinds of arthropods.

Another example of the specific adaptation between the various constituent parts of an individual is the following. Fresh serum or heparinized plasma from normal dogs as a rule causes a local reaction in capillary permeability when injected intradermally into other dogs, but this does not usually occur when the injection takes place into the dog from which it had been obtained (Freeman and Schecter). This is a further demonstration of an autogenous equilibrium. On the other hand, the presence of a species equilibrium is indicated, when, according to Togawa, injection of autogenous and homoiogenous serum causes an early increase in the amount of fibrinogen in the blood of the injected animal, while heterogenous serum usually does not have this effect.

All these observations have one characteristic in common: they illustrate a species or class or an autogenous equilibrium which latter we have analyzed previously. The various constituent parts of an individual organism are adapted to one another. Similarly the various components in the organization of a species or class are adapted to one another. In a previous chapter we have mentioned the fact that in more primitive organisms where it is not possible to demonstrate the existence of individuality differentials, species or class equilibria, indicating specific adaptations between the component parts of these organisms, may be present.

There exists a certain analogy between these specific adapations and the specific adaptation between the red corpuscles and presumably other cells belonging to a certain blood group and the serum of this group. In the latter case, the specific adaptation manifests itself by the lack of an agglutinating effect of the serum on the cells of the group to which both belong, although such a serum agglutinates the cells of individuals belonging to other blood groups which possess the necessary agglutinogen. But in this instance the specific adaptation is not due to a real species differential but to a special substance of a somewhat different nature. This relationship between serum and erythrocytes serves to maintain the autogenous equilibrium. Bernstein, however, interprets this phenomenon in a different manner; he assumes that the antigen (agglutinogen) of a certain group, circulating in the blood unites with [illegible]
fest, [illegible]
not being bound by this antigen, remain active. However, if this explanation

were correct, it would be difficult to understand why the equilibrum between antigen and agglutinin should be always balanced in such a way that no free agglutinin can be demonstrated in the blood. We know that in the case of toxin-antitoxin combinations such a perfect inactivation of either toxin or antitoxin is not possible. Moveover, a condition similar to that found in the case of the blood groups applies also to the Forssman antigens and the corresponding hemolysins. The blood sera of those species which belong to the heterophilic guinea pig group do not contain the hemolysin required for this reaction, while the sera of the species belonging to the nonheterophilic rabbit group do carry it. Now, in the case of the Forssman antigen, it can be shown that only animals belonging to the rabbit group can be immunized against the heterophilic antigen, while such antibodies cannot be produced experimentally in the heterophilic group. There must, therefore, be some mechanism which prevents the immunizing effect of antigen in the latter. These findings seem to be analogous to what we observe in the case of autogenous substances; they are not able to serve as antigens. Constituents of tissues are adapted in such a manner to the organism to which they belong that they cannot, here, call forth the production of antibodies; it seems, then, that the possession of the same individuality (or species) differentials on the part of antigen and receptive organs prevents the disequilibrium which is necessary for the production of artificial immunity. It is probable that this mechanism depends on the identity of the organismal differential proteins in an individual or species; in the latter, the species differential-proteins are the same, and in an individual the individual differential proteins are the same. Associated with and presumably superimposed upon these organismal differentials are the organ and tissue differential substances, which differ everywhere within the same individual. However in addition to the typical organismal differentials also other substances which help to maintain or play a role in the autogenous or species equilibrium may be adapted to the other parts of the organism in such a way that they cannot call forth the production of antibodies in the body to which they belong.

A species equilibrium can be recognized as mentioned already in a previous chapter in the observations of F. R. Lillie, who found that substances can be extracted from the eggs of various species, which possess an agglutinating power for the spermatozoa of their own species, but not for those of another species. Thus the egg extract of Nereis aggultinates the spermatozoa of Nereis, but not the spermatozoa of Arbacia. Similarly, the extract of eggs of Strongylocentrotus fransiscanus agglutinates the spermatozoa of the same species, but not those of Strongylocentrotus purpuratus, though the latter are agglutinated by the extracts of eggs of Strongylocentrotus purpuratus. However, such homoiogenous agglutinins cannot be demonstrated in the ova of all species, as Miss Sampson has shown. Heterogenous agglutinations may occur, but if this is the case, it is probable that the heterogenous agglutinins causing them are distinct from the typical homoiogenous agglutinins. There is another difference between the heterogenous and homoiogenous agglutinins; the agglutination produced by the former may be irrevers-

ible, and moreover, the heteroagglutinins may be toxic for the spermatozoa, whereas the agglutinations caused by homoioagglutinations are reversible and non-toxic for the spermatozoa (Little and Just). In addition, there has been found a more direct specific adaptation between eggs and spermatozoa, inasmuch as a smaller number of spermatozoa suffices for the fertilization of eggs of the same species than for that of eggs of other species (Jacques Loeb, R. F. Lillie).

A specifically adapted substance, which seems to be a protein, has been extracted from the sperm of the giant Keyhole limpet; it is able to dissolve the membrane of eggs of the same species. Correspondingly Abalone (Haliotis) sperm yields a lysin which acts on the eggs of Abalone; cross-lysis between limpet and Abalone does not occur (Tyler). In addition sperm extracts of Arbacia seem to agglutinate eggs of the same species, and this egg-agglutinating substance resists boiling for hours. Tyler has found that in certain echinoderms and worms there may occur in a watery extract of egg, a substance, fertilizin, which combines in a specific manner with the homoiogenous sperm, but without causing a noticeable agglutination of the spermatozoa. Such fertilizin he calls "univalent." There may be extracted from spermatozoa a similar species-specific substance, an antifertilizin, which combines with the fertilizin, neutralizes its sperm agglutinating power, and agglutinates the eggs from which the fertilizin can be extracted. Tyler noted moreover a certain relationship between fertilizin and the fourth component of complement which is present in normal guinea pigs serum. Complement is fixed by fertilizin, but is released from this combination by the action of antifertilizin; there exists thus a certain analogy between the action of complement and antifertilizin.

In a previous chapter we have referred to hormone-like substances which accelerate or induce metamorphosis in insects; also in amphibia there are indications of the existence of substances accelerating metamorphosis. In a similar way, Caswell Grave prepared from the larvæ of two ascidian species, Polyandrocarpa and Phallusia, extracts which induce metamorphosis in their own but not of the other species. These substances are therefore species-specific, yet they are neither proteins nor lipids; perhaps they are amino-acids; but their chemical nature has not been established.

According to F. B. Turck, a substance developing in autolysed muscle, or also in other tissues, has on certain cells very characteristic effects, which are either stimulating or injurious, according to the quantities used. This substance, which Turck names "cytost," seems to be species-specific. Thus, cytost from chicken acts specifically on chicken cells in tissue culture, and human cytost on human cells. Similarly, extract of dried paramæcia apparently stimulates the multiplication of paramæcia, while extracts from chicken, rat or human tissues do not have such an effect. Corresponding observations were made with bacteria. In immunization experiments it was shown that injection of autolysed muscle of the cat called forth active immunization only against cytost from the cat, but not against that prepared from other animals.

Specific adaptation may be found, furthermore, in the case of enzyme

action. There exist not only the first kinds of specificity, which imply that one enzyme is different from another one and is peculiar to a certain species or series of species and to a certain organ or tissue, but there has been demonstrated, also, a specific adaptation in the sense here defined. Thus according to E. N. Harvey, luciferin, the substance which, in being oxidized, gives rise to luminescence, if acted on by the oxydation accelerating enzyme luciferase, shows a specific adaptation to this enzyme. Only the enzymes from the same species, or from species very closely related to the species from which luciferin was obtained, seem to cause luminescence; if solutions of luciferin and luciferase are prepared from Cypridina and Systellaspis, the mixing of luciferin from one organism with the luciferase from the same species leads to a marked production of light; but if the solutions of luciferin from one species are mixed with the luciferase from the other species, the results are negative.

Another example from the field of enzyme activity is presented by certain older observations of Hedin. There occurs in the gastric mucosa of various vertebrates not only the milk-curdling enzyme rennet, but, according to Hedin, also a substance inhibiting the enzymatic action of rennet, which can be obtained if the enzyme is treated with NH_4OH. This inhibiting agent is specifically adapted to the enzyme of the same species, both of these substances, the enzyme as well as the inhibiting substance, carrying species differentials. However, certain other substances, such as egg albumin and blood serum, may also contain inhibiting substances for rennet, but they are non-specific; charcoal, likewise, may act in a non-specific manner. The species differential which is present in rennet participates in the antigenic function of this substance and calls forth in the animal, immunized against the rennet, the development of an anti-rennet, which is specifically adapted to rennet in a way similar to the natural anti-rennet. However, these investigations may perhaps have to be reconsidered in the light of more recent studies on proteinolytic enzymes of the gastro-intestinal tract. As far as the various enzymes and their precursors in the gastro-intestinal tract, which have been separated in recent years by Northrop and Kunitz, are concerned, it has been shown that their constitution differs in different species. Similarly, catalase seems to differ somewhat in different species (Sumner); also the urease which has been found in various tissues and in the blood serum of Limulus seems to be specific for this animal (Loeb and Bodansky). However, no instance of specific adaptation has been observed so far in these substances. Considerably more readily demonstrable than the species-specificity is the organ or "substance" specificity of these enzymes; each one is adapted to a definite type of substratum.

A specific adaptation is characteristic of many antigens and immune substances. In order to produce an antibody it is necessary to introduce into the organism which is to be immunized, a substance sufficiently strange to it to cause a certain disequilibrium. In many cases it is the introduction of a strange organismal differential which serves as antigen and makes possible the production of an antibody carrying the corresponding organismal differential.

It seems that specific adaptations of the kind mentioned here may underlie also some types of parasitism, the parasite becoming adapted to certain substances of the host which carry the species differential of the host, or at least differentiate one type of host from other types of hosts. Thus, according to J. H. Welsh, the freshwater mussel, Anodonta cataracta Say, is infested with parasitic water mites (Unionicola Ypsilophorus), which live between the gills of their host. In the free-living state these mites are positively heliotropic, but if to the water in which a positive heliotropic reaction would otherwise take place, an extract of the gills of the host or water from the mantle cavity of the host is added, they become negatively heliotropic, thus assuming the characteristic behavior they show in their parasitic life. It is interesting to note that in the case of Unionicola, which parasitizes on Anodonta, only material from this particular host will bring about such a change in behavior, whereas corresponding substances from other species, such as Ellipho or Lampsilis, have no effect on the parasite. In this instance we have to deal evidently with a specific adaptation between host and parasite, which depends upon the interaction of certain specific substances. However, whether the substances, which play the decisive role in these and certain other cases, actually carry the organismal differentials, or are merely derivatives of or otherwise related to these differentials, cannot be decided without further tests. But it could be made probable that the substances concerned in these reactions are at least nearly related to the organismal differentials of the parasite and host if, after immunization with these substances, the antibodies produced were found to react not only with the material which served as antigen, but also with other substances obtained from the same host species, but not from distant species; or, if it could be shown that there is a graded response of the parasite to analogous substances from different species, the response being the stronger the more nearly related the species from which the test substance is obtained, to the host species of that particular parasite.

In the examples which we have cited, we have to deal primarily with preformed relations between two substances, or between a substance and a tissue, the reaction depending upon the genetic relationship between the organismal differentials of the organisms concerned, although primarily, organs and tissues and organ-specific substances are involved in the majority of these reactions rather than purely individual and species-specific substances. The great structural and functional specificity which is characteristic especially of the higher, more differentiated organisms, depends largely upon this interaction of organismal differentials or of substances derived from them, or also of substances originating in organs which are specific for a species in a similar manner in which the organismal differentials are specific, but which differ otherwise from the latter. In addition, we have cited some instances in which, by means of artificial immunization, the same specific relations between different organisms, or between the substances derived from them, can be demonstrated in case one of the substances involved served as antigen.

These data may then be interpreted as indicating the presence of autogenous species or class equilibria, in which the various organs and substances

which are concerned in the function of these organs are specifically adapted to one another because they carry the same organismal differentials, and in which tissues or substances bearing strange organismal differentials call forth antagonistic reactions on the part of the host. However, in addition there may be active in this correlation between the phylogenetic relationship of animal species and the interaction of tissues and organs and of substances concerned in the functions of organs and tissues, other functions of tissues and organs, in which the organismal differentials are not involved and in which the active compounds may be of a less complex nature. However the finest and most varied examples of specific adaptations are furnished by the interactions between transplanted tissues and hosts which we have discussed in the chapters of the first part of this book. These experiments furnished also the basic data from which the concepts of organismal and individuality differentials and of autogenous tissue equilibria have developed.

Part VI Organismal Differentials and Organ Differentials as Antigens

Introductory Remarks

When pieces of organisms, organs or tissues, or when cells or parts of cells are transferred to or united with other organisms or parts of them, there are initiated those reactions which we have discussed in the preceding chapters, and which may serve as indicators of the nature of organismal and organ or tissue differentials. But in addition, the introduction of these tissues and cells, or of substances which are derived from them, may lead to the production of new substances and mechanisms which are specifically directed against the bearers of the organismal and organ differentials. These latter kinds of reactions represent immune processes and the altered state resulting in the strange organism is that of immunity; the specific substances formed in these reactions are immune substances or antibodies, and the substances which initiate these immune processes and lead to the development of immunity are antigens. Antigens and the corresponding antibodies may be considered as specifically adapted substances, which may either develop spontaneously or are produced experimentally.

In this part we shall discuss the relations between organismal and organ differentials and antigens. We shall also include in the discussion some substances which have certain characteristics in common with organismal or organ differentials, but which differ from them in some respects. There are, in addition, substances which are able to react in a specific way with antibodies, although unaided by proteins they may not be able to initiate immune processes, and therefore to act as complete antigens.

In the first chapter, we shall consider the differentials of blood groups and the heterogenetic (Forssman) antigens, which while differing in certain respects from the typical organismal and organ differentials, in some ways resemble them.

Chapter I

Blood Groups, Heterogenetic (Forssman) Antigens and Organismal Differentials

LANDSTEINER discovered, about forty years ago, that there can be distinguished in the human blood four groups of red corpuscles, according to the type of human serum which agglutinates them. Under normal conditions the serum of a person does not agglutinate the blood corpuscles of another person belonging to the same group, but the serum of individuals belonging to other groups has this power, except the serum of one group, which does not possess such agglutinating substances (agglutinins) for any of the blood groups. The red corpuscles of this latter group, on the other hand, contain both kinds of substances (agglutinogens) which are responsible for the agglutination of corpuscles under the influence of the specific group agglutinins in two of the groups. If the serum of this group possessed an active agglutinin, it would agglutinate its *own* blood corpuscles. There exists another group of individuals whose corpuscles cannot be agglutinated by the serum of any of the other groups, because their corpuscles lack both kinds of agglutinable substances (agglutinogens); correspondingly, their blood serum has agglutinins for all the other groups. Such agglutinogens, according to the terminology of Ehrlich's sidechain theory, are considered as receptors, which combine with the agglutinin to which they are specifically adapted and such a combination leads to the process of agglutination of the erythrocytes. Inasmuch as these agglutinogens, if injected parenterally into animals of a different species—e.g., the rabbit—may give rise to the formation of antibodies (immune agglutinins), acting specifically on the type of corpuscles which possess that particular agglutinogen which was injected, they may act also as antigens. In general, they represent the blood-group differentials.

We can thus distinguish four human blood groups, which differ according to the character of agglutinogens in their erythrocytes as well as according to the character of the agglutinins in their serum. In Group I, the corpuscles do not have any agglutinogens and in the serum there are found agglutinins alpha and beta. Agglutinin alpha has the power to agglutinate the corpuscles of Group II, and agglutinin beta agglutinates the corpuscles of Group III. In Group II, the corpuscles carry agglutinogen A and the serum agglutinin beta. In Group III, the corpuscles have agglutinogen B and the serum possesses agglutinin alpha. In Group IV the corpuscles have both agglutinogens A and B and, correspondingly, their serum lacks agglutinin alpha as well as beta.

As to the heterogenetic (Forssman) antigens or differentials, these are characterized by their ability to call forth the production of hemolysins for sheep corpuscles if they are injected into the rabbit. As a rule, only the injec-

tion of the red corpuscles of a certain species into a rabbit induces in the latter the formation of hemolysins specifically directed against the corpuscles of that particular species. But it has been found by Forssman that it is possible to produce hemolysins which dissolve sheep corpuscles not only by the introduction of sheep erythrocytes into a rabbit, but also by the use of kidney of the guinea pig or of the horse, or of the blood corpuscles of chicken, as antigens; if extracts of such cells or tissues are injected into rabbits, hemolysins for sheep corpuscles will be found to circulate in the rabbit blood. Similar differentials which may induce the formation of hemolysins for sheep corpuscles have been found in the tissues of the most diverse species of animals, and even in certain bacteria, without any reference to the relationship of these organisms with the sheep; but the organs of certain other species, such as the rabbit, do not usually possess such antigens. Accordingly, two classes of organisms are distinguished, namely those which, like the guinea pig, possess Forssman heterogenetic or heterophile differentials, and others which, like the rabbit, usually do not possess such differentials. The term "heterogenetic" is applied, because they are found in species and classes of animals far distant in relationship from the sheep, and even in bacteria. Evidently these substances behave in a very different way from organismal differentials; there is no specific connection between the systematic relationship of these organisms and the presence of the heterogenetic differentials in their cells, although the possession of Forssman antigens may be characteristic of whole genera and families. In addition to the Forssman antigens there exists still another system of heterogenetic antigens, which is shared by bacteria of the hemorrhagic septicemia group and the erythrocytes of many species of birds (Buchbinder), and presumably many other non-related groups have certain chemical characteristics in common. In this connection the fact may be recalled that also estrogenic substances occur in the most diverse classes of organisms.

In order to analyze the relationship of the blood-group and Forssman differentials to the organismal and organ differentials the following questions must be considered: (1) By what methods is the presence of the blood-group and Forssman differentials determined? (2) In which organs and tissues do these differentials occur? (3) What is the distribution of these differentials among animals and bacteria? and (4) What is the relationship of the blood-group differentials in various animal species to those in man?

Let us state once more the characteristic features of the organismal and, in particular, of the individuality differentials. In contradistinction to the structure and function of tissues and organs, which differ from one another, there is something common to all these different organs and tissues in the same individual, at least in the higher classes of animals, which differs from the corresponding characteristics in all other individuals. If we consider in addition, classes, orders, genera and species, and strains and family relationships, we then find that the various kinds of organismal differentials, including the individuality differentials, correspond in their graded properties to the graded phylogenetic relationships of these various types of organisms. This latter characteristic is very important in the definition of the organismal

differentials; it is not sufficient that certain structural or functional peculiarities should serve as distinguishing *marks* between different individuals or species, but a correspondence between the constitution of the organismal differentials and the genetic relationship of the organisms is required in addition. As we have seen, the sum of certain organ or tissue differentials, or even a single characteristic feature of a certain kind, may serve to distinguish different species as well as different individuals, but these individual organ and tissue differentials do not become thereby organismal and individuality differentials. Thus the ridge patterns of the skin, the scents and many other peculiarities, which are not individuality differentials, allow the differentiation between different individuals.

If we keep these criteria of the organismal differentials, and in particular of the individuality differentials, in mind, the differences which exist between blood-group differentials, their agglutinogens, and the organismal differentials are obvious. The primary blood-group differentials allow the separation of individuals into four groups, irrespective of their relationship. Two brothers, members of the white race, may belong to different blood groups, while one of the brothers and a member of an African race, or even an anthropoid ape, may belong to the same group. Thus the difference between individuality differentials and the differentials of blood groups is evident. Even the differentiation of individuals by means of the four primary blood groups is impossible as a general rule, although in certain cases they may help in identifying persons and even in establishing relationships to other persons; they resemble in this respect other hereditary organ characteristics, which may also be used for this purpose.

For the identification of the blood-group differentials we have at our disposal: (1) The various specific agglutinins normally present in human sera; and (2) the specific immune agglutinins which are produced by injecting, into rabbits, human blood corpuscles possessing a certain group differential, these immune agglutinins being absorbed in a specific manner by the agglutinogens (group differentials) to which they are adapted. Either the corpuscles as such, or alcohol extracts of the particular group of erythrocytes which contain the specific group differentials, are used for absorption. By these means we can determine also the occurrence of similar differentials which function as agglutinogens in blood corpuscles of various species of animals, or we may study the relationship of the blood-group differentials to other differentials, as for instance, the Forssman differentials.

The same principle applies to the analysis of the Forssman heterogenetic differentials, although in this case hemolysins, and in particular those dissolving sheep corpuscles, are used instead of agglutinins. Guinea pig or horse kidney, as bearers of the Forssman differentials, serves as tissue with which other material may be compared. In using these methods for the analysis of the identity or lack of identity between different kinds of differentials, we find that while certain differentials behave in every respect like the typical blood-group differentials, other differentials do so only in an imperfect manner. Results of this divergent kind are obtained especially when we study the blood-

group differentials in various species, or when we analyze the relationship between Forssman and blood-group differentials, and these results are interpreted as indicating that the various differentials have certain sidechains in common, while they differ in respect to others; or it is assumed that antigens with a blood-group, Forssman, species or organ differential, which are unlike in different individuals, are associated with other differentials (antigens) which are the same in two individuals and which explain the partial concordance in the results obtained in the testing of the antigens.

It is a very characteristic feature of the individuality differentials, and of the organismal differentials in general, that they occur in all or almost all of the various tissues and organs of a certain individual or species and are not restricted to one particular type of cell or tissue. At first it appeared as if the blood-group differentials were limited to the erythrocytes, but subsequently they have been found also in other cells, and according to Kritschewsky and Schwarzmann, they occur in all the organs of an individual, except the lens of the eye. The blood serum also seems to contain blood-group differentials, but here they are present in only a small quantity and are apparently covered up by other substances. They gained access to the body-fluid, presumably secondarily, perhaps as the result of the destruction of certain cells. As we have seen, also individuality-specific substances are present in the blood serum. In addition to the blood serum, various secretions, such as saliva and urine, may contain blood-group differentials. Landsteiner and Levine demonstrated "blood-group specific substances" in human spermatozoa, which had been freed from the sperm fluid through centrifugation and subsequent washing with salt solution. This observation suggests that germ cells contain preformed blood-group differentials; otherwise we should have to assume that some of the constituents of the sperm fluid may have adhered to the spermatozoa, or that a precursor substance of the fully developed differentials, rather than the latter themselves, was responsible for the group antigen reaction.

In regard to their general distribution among various tissues, blood-group antigens and organismal differentials behave, then, in a similar manner. As to the Forssman differentials, in one species they may occur only in the erythrocytes, in another species in the kidney, and perhaps also in the liver; in still others they may be found in the erythrocytes as well as in the kidney, and in the guinea pig they are present in the kidney, but only in the erythrocytes of certain individuals. In man, according to Schiff and Adelsberger, the Forssman differential is present in those corpuscles which possess the blood-group differential A; according to Kritschewsky, it is present also in various organs, but not in the brain. However, it is possible that blood-group A and the Forssman antigen have certain chemical characteristics in common, while they differ in respect to others; or there may be perhaps not even an identity of certain chemical groups, but merely a chemical similarity in these two antigens. This similarity in chemical structure may lead to an overlapping in the action of the resulting antibodies. In their wide distribution in human tissues the blood-group differentials would then differ from the typical organ differentials which, on the whole, are limited to one organ, although different

organs, such as liver and kidney, may have certain receptors in common; in this respect the blood-group differentials resemble the organismal differentials.

From what has been stated, it follows that by means of the four primary blood groups it is not possible, as a rule, to differentiate one individual from another, nor to indicate the degree of relationship between individuals. The behavior of transplanted tissues, on the other hand, does show not only the distinctiveness, but also the relationship of individuals in an approximately quantitative manner. All degrees of relationship are revealed by transplantation. This difference between the factors determining the results of transplantation and the differentials of blood groups among individuals belonging to the same, as well as to different species, is also emphasized by the lack of parallelism between the results of transplantation and blood-grouping. We have seen previously that the results of skin transplantation among human beings are not noticeably influenced by the blood groups to which these individuals belong. In animals transplantation reveals individual differences, although blood-group differences may be lacking here altogether. Furthermore, the presence of similar group differentials in different species of animals does not affect noticeably the severity of the reaction following heterotransplantation in these species.

What applies to the relations between the organismal differentials, as analysed by means of transplantation, and the human blood groups applies also, and to a still greater extent, to the relations between the heterogenetic differentials of Forssman and the organismal differentials. The Forssman differentials are in some respects the opposites of the organismal differentials; the latter correspond to and express the systematic relationship of organisms, whereas the Forssman differentials disregard these relationships; as stated they are factors held in common by the most varied and often distant kinds of organisms, without regard to systematic relationship. We may, perhaps, compare them in part with certain pigments which are present in the epidermis of the most varied species, without reference to their systematic position.

In many species of animals there occur in the blood corpuscles, species-specific agglutinogens, and in the blood serum, species-specific agglutinins, which latter cause agglutination of the blood corpuscles of foreign species, without reference to the blood-group to which they may belong. Inasmuch as these agglutinins are directed against heterogenous species, they are called heteroagglutinins; they are not, at least in some cases, experimentally or accidentally produced immune substances, but are preformed substances. At present it is not possible to establish a direct relationship between preformed heteroagglutinins and the organismal differentials, except that in some cases, when two species are relatively nearly related, heteroagglutinins seem to be lacking, as in the case of rat and mouse, or of buffalo and cattle; however, human serum may contain heteroagglutinins for the erythrocytes of nearly related anthropoid apes. In addition, there may occur in the serum of these species, hemolysins which are similar. Distinct from these preformed heteroagglutinins in the sera of various animal species are immune agglutinins

and hemolysins, which may be produced by injection of red corpuscles of one species into a strange species. These immune agglutinins and hemolysins also possess a species-specific character; the presence of such species-specific substances may obscure the existence of the group differentials, and in the case of immune agglutinins which are directed against human erythrocytes it may be necessary first to absorb the species-specific heteroagglutinins by human corpuscles of Group I, which possess neither the A nor the B group differentials, if a test is to be made of the presence of group agglutinins in this serum.

There exist, then, marked differences between the individuality differentials demonstrable by means of transplantation and the differentials of the four primary blood groups. It seems that it was the proof that very fine differences between individual constitutions can be established by means of transplantation which led immunologists to seek likewise for methods making possible finer differentiations between individuals by means of blood grouping. Accordingly, in recent years, by the use of immune agglutinins in addition to the natural blood-group agglutinins, Landsteiner succeeded in adding new groups to the four primary blood groups. Thus within the Group A, Landsteiner distinguished between two subgroups, A_1 and A_2; these differ in the way they unite with two subagglutinins, $alpha_1$ and $alpha_2$. In a somewhat related way Thomsen distinguished between the original Group A and the subgroup of the latter, A_1. A_1 corpuscles are less intensely agglutinated by antisera than are the typical A corpuscles. Thomsen thus adds to the differentials A, B and A+B, a fourth one, A_2. To these subgroups correspond subgroups among the agglutinins of the normal human sera.

Also, the B differential in human erythrocytes has recently been further differentiated into a B_1 component, which so far seems to be peculiar to human cells, and into B_2 and B_1, which occur, besides, in the blood corpuscles of certain animals, such as the rabbit. Correspondingly, anti-B of human sera may contain a mixture of anti-B_1 and anti-B_2; however, not all human sera contain the anti-B_1 component.

In addition, Landsteiner and his associates established three further subgroups carrying the agglutinogens M, N and P, respectively. No preformed agglutinins corresponding to the agglutinins M and N exist in normal human sera, but they can be produced through immunization of rabbits with these antigens. Moreover, in contradistinction to the primary blood-group antigens, M and N have not been found in cells other than the erythrocytes. These additional agglutinogens occur probably in all of the four primary blood groups and they, together with the ordinary blood groups and subgroups A_1 and A_2 differentials, make possible the differentiation between thirty-six classes of individuals. In this way the ability to differentiate between different individuals is much increased.

More recently, Schiff, through immunization of a sheep with the blood of a person belonging to Group O and possessing M, N and P differentials, established the existence of still another differential in human corpuscles, which he designates as H, and which may be present in any of the four primary human blood groups; it seems to be transmitted to the offspring by means of

a single dominant gene. Thus seventy-two classes of individuals can now be distinguished if one considers all these factors, and there is little doubt that the number of such differentials could be increased still further. Still more recently, the agglutinogen Rh, which is common to man and the Rhesus monkey, has been added to the list of blood-group antigens.

Notwithstanding the possibility of finer differentiations of individuals by such means, these blood-group differentials are not identical with the individuality differentials, according to the evidence which is available at the present time. The fact that two individuals belong to the same primary blood group does not seem to have any relation to the reaction which takes place if a piece of skin is transplanted from the one to the other. Furthermore, inasmuch as A_1 and A_2 represent subgroups of A, the same objection applies to the identification of these subgroups with the individuality differentials as to the primary group A. In regard to the M, N and P differentials, they are apparently inherited in a similar manner to the four primary blood groups; neither they nor H, as such, would make possible a differentiation between different individuals. But even if it should be possible to distinguish individuals by means of these additional blood groups, it has not been shown that the mode of distribution of blood-group differentials among the different individuals corresponds to their degree of relationship, and even if contrary to expectation there should be found such a parallelism, it would still remain improbable that these differentials are identical with the individuality differentials so generally found among all kinds of species and animals, including those in which these particular blood-group differentials are lacking.

In addition to the secondary blood group or subgroup differentials, there occur other unusual agglutinogens and agglutinins in the blood of various individuals, or in certain classes of individuals. Several authors—Guthrie and Huck, Ottenberg and Johnson, and others—have already drawn attention to such occurrences. Thus it seems that especially in cases of insanity abnormal agglutination reactions have been observed. Furthermore, if the union between agglutinogen and agglutinin takes place at a low temperature, abnormal agglutinations may result, which are not found at ordinary temperature. Other complications are due to an apparent linkage which has been noted between certain types of agglutinogens or agglutinins. Thus an agglutinin for A_2 usually causes an agglutination also of blood corpuscles which belong to the primary blood group I, possessing neither A nor B. Agglutinin $alpha_1$ of human sera from groups I and III can be removed by blood cells of Group A, which lack the A_1 receptor. It is possible that with the agglutinogens N and P, there may be associated other agglutinogens which increase the agglutination effect normally produced by the union of N and P and their respective agglutinins; perhaps anti-human species agglutinins may be active in anti-N or anti-P rabbit immune sera and cause agglutination in addition to the specific agglutination of the N and P corpuscles. Agglutinins for human P agglutinogen have been observed also in sera of horses, hogs and rabbits. Another indication of the complexity of this mosaic of antigens is the existence

of a common partial antigen in human corpuscles, and in other cells, without respect to the group to which they belong, as well as in certain Shiga bacilli; and in the latter, in addition, the Forssman antigen is present. There are a number of other differentials which are found in cells of various species, irrespective of their systematic relationship. Thus human corpuscles of Group A have a certain factor in common with erythrocytes of hog, sheep and cattle. Besides, common differentials are present in hog erythrocytes and in the erythrocytes of man, regardless of the blood-grouping of the latter. There are known still other differentials, which different, not phylogenetically related, species have in common, and in all probability a still larger number, unknown as yet, could be added to those which have so far been established.

All these observations exclude the possibility of identification of these agglutinable factors with organismal differentials, but not the possibility that these various factors, or some of them, may be present among the individuality differentials, or, rather, that the genes representing these factors may be a component part of the gene sets which determine the individuality differentials. This conclusion holds good even if it should be feasible to distinguish all individuals by a study of their blood-group antigens. If we consider that besides the differences already established between human corpuscles, or between the blood sera of human groups, and of certain individuals in these groups, additional differences might be discovered between the red corpuscles of individuals if their reactions with different animal sera were studied, then we can conceive the possibility that, as Landsteiner suggests, in this way the corpuscles of all, or at least a large number of individuals, might be identified and distinguished from one another. Yet, as stated, it would not follow therefore that the sum of these factors constitutes the individuality differential. This would require that there should be found the same graded relationship between these regular or irregular, often heterogenous group differentials and the genetic constitution of individuals, as can be shown to exist in the case of the individuality differentials. That an individual can be distinguished from other individuals by means of certain characteristics does not, by itself, prove that these characters represent the individuality differentials. We may be able to identify an individual by means of the combination of certain characteristics which are a part of the Mendelian mosaic of this individual, or even by a single characteristic belonging to the Mendelian mosaic. It is the organismal differentials which determine the graded compatibility in the biological sense between two organisms, and especially between two individuals of the same species or race, whereas the blood groups and the immune reactions based on such group differentials, which may be common to man and nearly, or even more distantly, related species of animals, do not exert this function as far as known at the present time. We have discussed these problems already in a preceding chapter, where we considered the relation between natural and experimentally produced immune hemolysins in cattle and fowl and organismal differentials, and we concluded that certain relations may exist between these types of substances. However, it may be assumed that

the greater the number of additional antigens which will be found in the erythrocytes, the less will be the difference remaining between the totality of *these* antigens in the erythrocytes and in the individuality differentials.

Investigations as to the distribution of blood groups in different human races we owe especially to Hirszfeld and his collaborators. In general, it may be stated that the different primary blood groups are found in all races, but the frequency with which the different groups occur differs in different populations and races. In general, in Western Europe A predominates; the farther we progress in the direction of India, the greater the frequency of B. In certain more primitive races, such as the American Indian and the Eskimo, O is the most common blood group; but among the Black Feet Indians, Matson and Schrader found a marked preponderance of Group A. As a rule, among whites and negroes a certain agglutinogen may occur with varying frequency.

From these facts it may be concluded that the differences even between supposedly pure races are essentially statistical as far as their blood-group differentials are concerned. On the other hand, as stated above, different species may possess the same kinds of blood groups. Conditions are different in transplantation. Here a large number of very fine gradations in reactions occurs in accordance with the relationship of donor and host. If we compare, for instance, the results of transplantations of thyroid from rat to mouse, or of the reciprocal transplantations, with those of syngenesio- and homoiotransplantations in rats, we do not find in the former, in a single instance, the excellent state of preservation of the thyroid transplant which may be observed in a favorable syngenesiotransplantation from rat to rat; we have here to deal with absolute differences in the distribution of a large number of factors, not merely with statistical differences in the distribution of a limited number of factors as in the case of the four blood groups. In homoio- and inter-racial transplantations we may find an overlapping of the results; but the most favorable ones obtained in some syngenesiotransplantations are not observed in inter-racial transplantations; the differences in the results of different kinds of transplantation are therefore not merely of a statistical nature, such as those obtained in comparing the blood groups in different populations.

In order to analyze further the relations between blood-group differentials and organismal differentials, we shall consider the occurrence of the former in the cells and of agglutinins in the blood serum in different species of animals. However, some of the evidence concerning this subject is still contradictory and the data on hand must, therefore, be used with caution.

There can be no doubt that in certain anthropoid apes, as Landsteiner and Miller have shown, there are group differentials which are identical with those in man. Thus A and O corpuscles occur in chimpanzees, and A, B and AB corpuscles in orang-utangs. In a Gibbon, A corpuscles were found. In the blood serum of these species there are present agglutinins which react with those differentials not present in their own erythrocytes. The agglutinogens and agglutinins in these anthropoid apes behave exactly like the corresponding

human group differentials and agglutinins. This relationship of the group differentials corresponds to the close relationship existing between the organismal differentials of man and these apes. However, notwithstanding the identity of group differentials in these organisms, a very marked difference exists as far as the organismal differentials in man and anthropoid apes are concerned. Injection of human red corpuscles into chimpanzees leads to the production of species-specific antibodies, which allow the differentiation between human and chimpanzee blood (Landsteiner and Levine), although both of these may possess the same blood-group differentials. This fact again demonstrates the distinction between organismal and the original blood-group differentials. The combination of blood-group antigens M and N has been detected so far only in the blood of primates, but the M differential seems to occur also in the Macacus Rhesus erythrocytes.

If we turn to the lower monkeys, Landsteiner and Miller found among Old World monkeys no blood-group differentials which correspond to human isoagglutinogens, while New World monkeys, which are less nearly related to anthropoids than Old World monkeys, have a differential analogous to human differential B, although B of man and monkey are not identical in this instance. Among the Old World monkeys it is especially Macacus Rhesus that has been studied very extensively. According to Buchbinder, the erythrocytes of Macacus do not possess a differential corresponding to the human blood-group differentials, but in the blood serum of this species there is found the isoagglutinin alpha, which agglutinates human corpuscles A. However, more recently it has been observed that antigen Rh is common to human and Macacus Rhesus erythrocytes. No Forssman differential exists in Macacus erythrocytes or kidney, but there is a hemolysin for sheep corpuscles in Macacus serum; however, this hemolysin seems not to behave in the expected way towards the corpuscles of other species which contain Forssman differentials. Macacus erythrocytes do not contain blood-group differentials A and B, and no classification of Macacus blood into groups is possible. As Eisler has found, human corpuscles have also a differential distinct from the Forssman differential, in common with Shiga bacilli.

We see, then, that to a certain extent the blood-group distribution is connected with the phylogenetic relationship of animals; the anthropoid apes have blood groups more similar to those of man than the lower monkeys and other animals. However, this is a condition which is not restricted to blood groups, but which is observed likewise in other organ characteristics; thus the shape of the skull and brain, and many other features, are in apes, more similar to those of man than are those of other animals. On the other hand, this parallelism between relationship and blood-group distribution is not general and, moreover, we find quite similar characters shown equally by very diverse organisms, without respect to their relationship.

The investigations in apes and monkeys were preceded by those concerning the blood groups in other mammals. At first the same methods which had led to their establishment in man were used also in the case of various animal

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of this substance may pass from the organs into the serum. In those rabbits which contain the A differential, the anti-A agglutinin is lacking in the serum; on the other hand, the anti-A agglutinin can be demonstrated in the serum of those individuals in which the A differential is not present in their organs (W. Treibman). The latter type of animals can be immunized against the human A differential, which is strange to them; while the former type, which possesses the differential, cannot thus be immunized. These observations indicate very strongly that the A differential occurring in the organs and serum of certain individual rabbits, is essentially the same as that occurring in the erythrocytes of man.

In a corresponding manner, according to Hirszfeld and Halber (1928), the isoagglutinable substance of sheep and hog is serologically identical with the isoagglutinable substance A of human blood. The isoagglutinable blood corpuscles of certain sheep and hogs absorb all the anti-serum A antibodies obtained through immunization, whereas the non-agglutinable blood corpuscles of other sheep and hogs do not possess this property. Therefore, according to these investigators, we must conclude that sheep and hogs actually possess an A differential identical with that of man.

Still, certain differences seem to exist between the A differential in human corpuscles of blood-group II and in those sheep corpuscles which also possess A. While by means of absorption with A-containing sheep corpuscles it is possible to absorb more antibodies from the serum of rabbits immunized against the human A differential, than by using for this purpose sheep corpuscles which do not contain the A differential, on the other hand, if the sera of rabbits immunized against A-containing sheep corpuscles are absorbed with A sheep corpuscles, then all antibodies against the A differential of sheep corpuscles are entirely removed, but only a part of the antibodies against human corpuscles of group A is removed. Similarly, from serum of rabbits immunized against human differential A, the antibody against the A differential of sheep corpuscles can be entirely removed through absorption with sheep corpuscles containing A, but there remains, then, still a reaction against an alcohol extract from human A corpuscles. These observations have led to the conclusion that there exists not a complete, but only a partial identity between the A differential in the erythrocytes of man and of sheep; but we may possibly have to deal merely with quantitative differences between the A differential as it occurs in man and in certain animal species.

While no isoagglutinable substance was found by direct test in Polish cattle, indirectly a grouping could be demonstrated in these cattle erythrocytes. Cattle and sheep corpuscles have a receptor in common, as is shown by the fact that the amboceptor in the serum of rabbits immunized against sheep corpuscles hemolyzes not only sheep but also cattle corpuscles, whereas the anti-Forssman serum, obtained through injection of guinea pig kidney into rabbit, which also hemolyzes sheep corpuscles, does not affect cattle corpuscles. Therefore the antigen in sheep corpuscles which gives rise to the formation of antibodies able to act on certain cattle corpuscles, is not identical with the

blood corpuscles and sera. Employing these methods Hektoen obtained negative results, but von Dungern and Hirszfeld reported some which were positive, although the reactions in these animals were weaker and more irregular than in man. The conclusions became more definite when not only blood corpuscles and sera from individuals belonging to a certain species of animals were compared, but when, in addition, the interactions between sera and blood corpuscles of these animals with the well defined human agglutinogens and agglutinins were studied; and furthermore, when use was made of immune sera, obtained in rabbits by injection of human or animal blood corpuscles, and when comparative absorptions of the antibodies, present in the immune sera, by human as well as by animal erythrocytes or their alcohol extracts were also considered. By these means the identity of certain group agglutinogens in human erythrocytes and in the erythrocytes of more remote animal species has apparently been demonstrated, as well as the identity of certain agglutinins in animal and human sera, while other blood-group differentials and agglutinins have been found to be limited to man or to various species of animals.

However, in some instances it has been possible to establish the presence of blood groups in animal species by the same methods which have been used for this purpose in man. Thus Hirszfeld and Przesmycki, and also Schermer and Hofferber, have shown that in the horse four groups exist, which are analogous to those in man, namely, O-alpha, beta, A-beta, B-alpha, and AB-oo. The similarity between the blood groups of man and horse goes still further. Thus in both of these species, analogous subgroups A_1 and A_2, and two agglutinins, $alpha_1$ and $alpha_2$, can be recognized; in both instances the differences between these subgroups are presumably of a quantitative rather than a qualitative character. Furthermore, in addition to the primary four blood groups, four additional blood groups, X, Y, Z and N, comparable to the additional blood groups M, N, P and H in man, are demonstrable in horses (Schermer and Kaempffer).

Similarly, by means of injections of rabbits with the erythrocytes from other rabbits, Fischer and Klinckhard prepared immune sera which agglutinated blood corpuscles from certain groups of rabbits. They believed they were able in this way to establish the existence of two agglutinogens and two agglutinins, and they divided therefore these animals into four groups, corresponding to the four groups found in man, although neither agglutinogens nor agglutinins were identical with those of man. However, Levine and Landsteiner, by immunizing rabbits with the hemolyzed blood corpuscles of other rabbits, obtained a larger number of agglutinins, and they assume therefore the occurrence of individual blood differences in rabbits similar to those which have been established in goats, cattle and chickens, and which we shall discuss in a subsequent chapter. But there occurs in certain rabbits a condition which differs from the usual findings in man and in other animals. There may be observed in these particular animals a peculiar distribution of the A differential; it is lacking in their erythrocytes but is present in their organs, and some

Landsteiner, Miller and Levine, and we shall refer to them again in a later chapter, where we shall discuss the use of serological methods in the establishment of individual differences in several species of animals.

Various phases may be distinguished in these investigations into the occurrence of blood-group differentials and agglutinins in human and animal cells and sera. At first it appeared as though the blood-group differentials in man and in certain animals were identical; then certain differences were found and doubts arose as to whether the identity was complete. Thus von Dungern and Hirszfeld observed that human beta sera are not only absorbed by human B corpuscles, but also by B corpuscles of various animal species. But subsequently it was discovered that the beta agglutinin of these animal sera cannot be absorbed in a corresponding manner by B human and animal corpuscles. Moreover, from human anti-B immune rabbit serum only B of human origin and B from some anthropoid apes can absorb the B agglutinin; whereas the B corpuscles from other animal species cannot do so. Further investigations made it then very probable that the agglutinogens A and B can be subdivided into various fractions and that, correspondingly, the different serum agglutinins can be subdivided; also, that certain of these secondary differentials may be common to human blood and that of various animal species, while others are peculiar to single species. The question now arises as to how far it is possible to proceed with this process of subdividing corpuscles and sera; it is not improbable that by increasing the number of tests between corpuscles and sera of man and those of different types of immune sera against a greater variety of blood corpuscles, employing other species than the rabbit as donor of the immune sera, the number of subdivisions may be still further augmented. In addition the question suggests itself as to whether experiments of this type actually prove the existence of multiple differentials and agglutinins, or whether we have to deal merely with quantitative differences in the strength of different differentials and agglutinins and with the presence of substances which may interfere with the absorption processes in different types of blood.

We have seen, then, that various species of animals, including the human species, have certain differentials in common, without regard to the relationship of these species. In addition, a connection has been established between blood-group differentials and differentials of different kinds. Thus a relation has been noted between the human blood-group differential A and the Forssman differential of sheep erythrocytes, guinea pig kidney, and certain cells of other heterogenetic species, and even of some bacteria. Schiff and Adelsberger first found that through immunization of rabbits with human erythrocytes A, hemolysins for sheep corpuscles are produced, as well as immune agglutinins for the human corpuscles of Group A. As to whether differential A and the Forssman differential are identical, or whether they occur side by side in the same human corpuscles, it seems, according to investigations of Sachs and Witebsky, that the second alternative holds good. It can be shown that some sheep corpuscles which possess Forssman antigen—as well as other sheep corpuscles which do not possess it—have no human A

Forssman differential, which likewise occurs in sheep corpuscles. But the antigen common to sheep and cattle corpuscles which gives rise to this hemolytic amboceptor is not possessed by all cattle, but only by some individuals. Thus the presence of groups can be demonstrated also in the case of cattle corpuscles. Cattle contain B_1 and B_2 receptors. B_1 is the differential of those blood corpuscles which are not hemolyzed by anti-sheep-rabbit serum, while B_2 cattle corpuscles are hemolyzed by anti-sheep-rabbit serum.

In general, in lower animals the agglutinogen B apparently is more frequent than A. Thus in the dog, in the rabbit, and, as mentioned above, also in New World monkeys, B is present, and correspondingly the agglutinin alpha is found in the serum of such individuals; also in the guinea pig a very weak B has been noted. More recently the occurrence of B differentials in erythrocytes and of anti-B in sera has been further analyzed by Friedenreich and With. It was found that among the B differentials, different subgroups can be distinguished, namely B_1, B_2 and B_3. The separation of these fractions was accomplished by means of absorptions of normal human and animal sera by human B corpuscles, as well as by B corpuscles from various animal species. Rabbit corpuscles were observed to contain B_2 and B_3 differentials, but not B_1, which is peculiar to man. The guinea pig erythrocyte has a weak B differential; also, dog, rat and hog have B components. The same facts apply to cattle, sheep and goats, although their erythrocytes are not agglutinated by anti-B serum. In conformity with the lack of B_1 in the erythrocytes of these various species, the sera of the latter possess anti-B_1 substance; only the chicken serum shows anti-B substance, and correspondingly, the chicken erythrocytes are free of the B differential. That different kinds of B differential may be distinguished explains also the occurrence, in some species, of B in the red blood corpuscles and of agglutinin beta in the serum of the same individual; in such cases, B and beta represent different fractions, as for instance, B_2 in the corpuscles and anti-B_1 in the serum. In this way auto-agglutination would be avoided.

In Polish chickens the sera of many individuals strongly agglutinate human erythrocytes O and B, but not A. Conversely, according to Karshner, human serum belonging to blood-group B-alpha gives the greatest number of positive agglutinations with chicken erythrocytes, while human sera of blood-group A, containing anti-B agglutinin, give the least. Dunn and Landsteiner, by means of anti-chicken-rabbit sera found in several chicken families an agglutinogen, the hereditary transmission of which was apparently determined by a single dominant gene. Karshner, by means of isoagglutination reactions, distinguished three blood groups in chickens, the largest group consisting of individuals in which neither agglutinogens nor agglutinins could be demonstrated. On the other hand, Shimidzu did not find that the weak isoagglutinations which occur in chickens permit the differentiation of different groups. However, if the agglutination of the erythrocytes of individual chickens is tested with rabbit-anti-chicken immune sera, or with various heterogenous sera, individual differences in the majority of all individuals examined could be found. Such experiments were carried out by

In regard to the time of development of the blood-group differentials and of the corresponding agglutinins in the blood serum, this shows some parallelism to the time of origin of the organismal differentials. The experiments of Blumenthal have shown that the latter are not yet fully developed during the first stages of embryonal development, but can be demonstrated during the second half of the intrauterine life of the embryo. From Murphy's experiments on the transplantation of heterogenous tissues to the allantois, and from similar experiments of various embryologists, we may conclude that in early embryos the species differentials, or rather certain mechanisms of reaction against such differentials, are not yet developed, and also that the bodyfluids are not yet injurious to the strange transplant, but that not very much later the harmful mechanisms develop in the embryo. However, as we have seen, even during post-embryonal life the reactions against strange individuality differentials are not as strong in very young animals as in adults. In all these cases it is necessary to distinguish between the presence of the organismal differentials and of the reactions against the latter on the part of the strange organism. The reactions may not yet be fully active at a time when the differentials have already been completely formed.

One of the characteristic features of individuality differentials, and organismal differentials in general, is the lack of an injurious reaction of the bodyfluids as well as of the cells of the host against cells or tissues which are derived from the same organism, and which possess therefore the same individuality and the various other organismal differentials. This fact is expressed in our definition of individuality and organismal differentials. Similarly, it is well-known that, as a rule, it is not possible to produce antibodies against autogenous normal cells, or against substances which represent a normal constituent of the animal to be immunized, especially if it is accessible to his bodyfluids. This applies also to the Forssman differentials, against which antibodies can be produced only in those species which do not possess this differential; and the same holds good presumably in the case of the blood-group differentials. It is a very interesting fact that if the blood cells of an individual contain a certain agglutinogen, his blood serum lacks that particular agglutinin which would interact with its own blood corpuscles and cause their agglutination, and that thus the formation of agglutination thrombi and emboli is avoided (Landsteiner). As we have discussed in an earlier chapter Bernstein assumes that in every individual, to whatever blood group he may belong, all the agglutinins are produced, including those which are able to agglutinate his own corpuscles; but the latter kind of agglutinins are made innocuous by union with the corresponding agglutinogens present in the erythrocytes of the same organism, and only agglutinins which would act on corpuscles belonging to other groups are left intact. Against this interpretation may be cited the observation that injection of rabbits with human blood corpuscles of group A seems to cause the formation of anti-A immune agglutinins only if the serum of these rabbits contains normally some anti-A antibodies, which implies that the cells of this animal do not contain A receptors. But if actually the A agglutinin was able to develop in a rabbit which possesses A

antigen; they absorb only traces of the human anti-A substance preformed in human serum. This indicates that the Forssman differential and the human A differential are not identical.

In anti-human A rabbit immune serum which, as stated, contains also Forssman antibodies, it is possible to remove the latter by absorption with sheep corpuscles which contain the Forssman differential but do not contain the human A differential; at the same time the ability of the immune serum to react with alcohol extract from human corpuscles A is also diminished by the removal of the Forssman antibodies. Forssman antibodies and antibodies against human A differentials are perhaps in some loose manner linked, and a certain constituent of the Forssman differential may occur in human corpuscles belonging to group A. However, it may also be that the common reactions of group A and Forssman differentials depend merely upon a similarity in their chemical structure.

In the case of the organismal differentials we have seen that the reactions against strange differentials are not yet fully formed in very young organisms. The development of the human blood-group differentials seems to set in at about six or seven months of embryonal life and to be completed at the time of birth. But it has been maintained that the full development of the A differential occurs only at the age of fifteen to twenty years.

As to the agglutinins A and B which circulate in the blood serum, these originate within the last two months of pregnancy; they develop therefore later than the differentials present in the cells. In some cases they seem to be lacking at the time of birth and to be formed only in the first few months of extrauterine life. According to Thomsen, they reach their full development only in children between five and ten years of age, and a decrease in the quantity of these agglutinins may occur in old age; but this age involution may take place fairly early and may therefore be found even in relatively young individuals (Schiff and Mendlowitsch).

If agglutinins are found in the blood of the newborn child, they may have been derived from the mother, having reached the foetus by way of the placenta. But in case mother and child belong to different blood groups, no pathological effects seem to result from the combination of agglutinins in the blood serum and the agglutinogens in the erythrocytes, which, theoretically, should be expected to act on each other. It is assumed that mechanisms exist which, as a rule, prevent the passing through the placenta of maternal agglutinins capable of agglutinating the erythrocytes of the child. Von Oettingen and Witebsky believed that the occurrence of the blood-group differentials could not be demonstrated in the embryonal part of the placenta, although they are found in the maternal decidua. According to Kritschewsky, it is the Forssman differential which is present in the decidua and not the blood-group differential, while the embryonal placenta is free of the latter. However, Levine has found that the Rh antigen may pass in the uterus from the child to the mother. If the latter does not possess this antigen, antibodies may be produced against it, which then pass in the opposite direction from the mother to the child and here may cause erythroblastosis foetalis.

only from that of the individuality differentials, but also from that of the accessory blood-group differentials.

There are found, thus, in human erythrocytes, a number of different differentials. We have mentioned the occurrence of species differentials, of the typical blood-group differentials, of the accessory blood-group differentials, of the Forssman differentials, and of certain special differentials which human erythrocytes and erythrocytes of some distant animal species have in common. In addition, we must consider the possibility of the occurrence of organ differentials in various types of human cells. In this connection the observations of Jacobs are of interest. He compared the ability of the erythrocytes of many different species to absorb various kinds of chemicals and he found, on the whole, that the corpuscles of related species resemble each other more in their permeability to and absorbing powers of certain substances than do the erythrocytes from animals more distant phylogenetically, although a strict grading according to phylogenetic relationship is not possible. The red blood corpuscles behave in this respect like some blood-group differentials and characteristics of organs which may show a certain correspondence to the phylogenetic development of these cells and organs. But a gradation in the organismal differentials present in the erythrocytes according to the relationship of the various species would also explain this phenomenon.

This is in all probability a very incomplete list of the differentials occurring in erythrocytes and we may assume that besides those named, there occur other differentials. Of special interest for us is the question whether also individuality differentials are present in human erythrocytes. Experiments which we have discussed in an earlier chapter make this very probable. Do transfusion experiments give any indication of their presence? There are some observations concerning injurious results following transfusions of apparently compatible blood which suggest such a possibility; but other interpretations of these occurrences, such as the presence of an agglutinogen common to man and Rhesus monkey in the blood of the donor, or of an immune agglutinin in the blood of the person which received the transfusion, or the presence of agglutinogens A and B in human blood plasma used for intravenous injection (M. Levine and D. State), cannot be excluded.

We shall now briefly summarize our conclusions concerning (1) the relations between blood groups and organismal and, in particular, individuality differentials; (2) the relations between blood groups and what is designated as "constitution," and (3) the general significance of the antigens discussed in this chapter.

(1) The primary blood-group differentials do not make possible a distinction between different individuals in general, but only between some individuals; they do not indicate the degree of relationship between individuals; they differ in these and in other respects from the individuality differentials.

However, if in addition to the primary blood-group differentials, we consider other types of differentials which may be found in erythrocytes, such as the accessory blood-group differentials, the Forssman and accessory hetero-

agglutinogens and it was merely hidden by its union with these agglutinogens, then it should be possible by means of an intense immunization to produce enough anti-A agglutinin to overbalance the slight amount of A receptor present in the blood plasma. Or if it is assumed that the red blood corpuscles themselves can bind and inactivate this immune agglutinin, then an agglutination of the erythrocytes should take place in the immunized animal. Such an effect however seems not to have been observed. Similarly, as mentioned already, antibodies for the Forssman antigen cannot be produced in animals which belong to a Forssman positive species, although in this case erythrocytes do not need to contain the heterophile differential.

Taking these various considerations together, we think it much more probable that in the case of the blood groups we have to deal with the same phenomenon as in the case of the organismal differentials, namely, that in the same organism mutually incompatible constituents do not develop, and that the normal constituents within the body, especially if they are present also in the bodyfluids, cannot serve as antigens, and that this is due to the fact that in the same organism the analogous proteins and, in particular, certain globulins possess some essential similarity in chemical structure irrespective of their situation in the individual. The production of antibodies can take place, as a rule, only when these differentials show a definite divergence in chemical constitution in host and donor.

Organismal differentials, primary blood-group differentials, the more recently discovered accessory differentials occurring in human and also in certain animal erythrocytes, as well as the heterophile Forssman differentials, all have this characteristic in common, that they are genetically fixed constituents of the various organisms and do not owe their origin to environmental factors. As to the mode of inheritance, there are differences between the organismal, and in particular, the individuality differentials and the primary as well as the accessory blood-group differentials. The individuality differentials depend, as we have discussed previously, upon the presence of multiple factors, the number of which must be considerable. On the other hand, the inheritance of the primary blood-group differentials seems to depend upon three allelomorph factors, according to the analysis of human inheritance by Bernstein, whose interpretation has now been almost generally accepted. The inheritance of the primary blood-group differentials follows therefore a much simpler scheme than the inheritance of the individuality differentials. Among the latter many fine gradations exist, while among the former there is only a small number of variables. According to Landsteiner, Schiff, and other investigators, the inheritance of the accessory factors M and N is contingent on the presence of an allelomorph pair of genes. The possible combinations of these two genes are M M, N N and M N. The factor P also seems to be fixed by heredity. It has been observed that when neither of the parents contains P, none of the children contain it. The factor H is believed to be represented by a single dominant gene. We may then conclude that the mode of inheritance of the primary blood-group differentials differs not

ditions, may serve to distinguish between individuals, and inasmuch as these characteristics have a genetic basis, they might in a limited way even indicate certain relationships between individuals. But they would not, therefore, become identical with individuality differentials. The four primary blood-group differentials are essentially tissue differentials, which have however certain characteristics in common with the individuality differentials while they differ from the latter in other respects; but the larger the number of accessory blood-group differentials which are added to the primary group, the greater will, in all probability, become the similarity between blood-group and individuality differentials.

(2) Some authors have identified the blood-group differentials with the constitutional characteristics of a certain individual. The constitution of an individual means an inherited or acquired constellation of structures which determine his characteristic modes of reaction or tendencies, including those of an abnormal kind. These inherited or acquired modes of reaction or tendencies, as a rule, become manifest only in their interaction with variable factors of the inner and outer environment. The emphasis is laid in this definition of constitution on the reaction-modes; but constitution may also mean that a certain reaction-mode of an organism is associated with a specific inherited habitus or structural feature, and in this case the emphasis is laid on the structural aspect. Only in the sense that the blood-group differentials are an inherited characteristic may they be considered as part of the constitution, without, however, representing the whole or even the essential features of the constitution. Probably because of the readiness and sharpness with which the blood-group differentials can usually be determined in human beings, and because of the role they play in blood transfusion and because of the inherited differences in their distribution among different individuals were they considered as specially representative of the constitution of individuals or races. However, there is more justification for the belief that the various kinds of organismal differentials represent to a much higher degree the constitution of an individual, or a race, or of a species, than do the blood-group differentials.

(3) Individuals and species may have special genes or gene combinations in common which determine the formation of special differentials and antigens as revealed by serological methods. The latter indicate particular relationships between these individuals and species or they can be used as a means of distinguishing between these individuals and species. The main problem which we have discussed in this chapter concerns the connection between such special differentials and the organismal and in particular the individuality differentials and the relationships which the various differentials have to one another which may render them significant in the analysis of individuality.

genetic antigens, it is possible that all of them combined, or even a certain number of them, might suffice to distinguish individuals, but they would not in all probability indicate the relationship of these individuals. But there is reason for assuming that also a combination of various organ differentials might differentiate between different individuals, although they would not be identical with the individuality differentials. To demonstrate the identity of the former with the individuality differentials, the proof would first have to be given that they are qualitatively the same in all the essential tissues of the same individual, and different in all other individuals; furthermore, that these sets of blood-group differentials are actually the ones which function in the various individuals as individuality differentials, or that they are an important constituent of the individuality and species differentials. This is very improbable as far as the primary blood groups are concerned, because, as we have seen, the results of homoiotransplantations are independent of the distribution of these differentials; besides, the presence of similar blood-group differentials in different species of animals does not affect noticeably the severity of the reaction following heterotransplantation in such species. The Forssman antigens, which are characteristic of very diverse species, can be removed or neutralized by means of specific absorption without the species differentials being affected by this procedure. However, as already stated, if the number of blood-group differentials serving as agglutinogens or able to give rise to the production of hemolysins is greatly increased in the individuals belonging to a certain species, then it is possible that such sets of potential antigens may more and more coincide with the factors composing the individuality differentials.

To recapitulate: There are at least four requirements which have to be satisfied before a set of differentials can be accepted as representing the individuality differentials: (a) they must make possible the distinction between individuals; (b) they must occur in all or almost all the tissues of an individual and thus allow the distinction of the tissues of one individual from the different tissues of another individual; (c) they must indicate the relationship of a particular individual to another individual, and (d) their inheritance must not depend upon a very small number of factors which are transmitted in accordance with simple Mendelian rules of alternate inheritance. The primary blood groups, and even the accessory blood-group differentials, as well as the various heterogenetic antigens have not, so far, been shown fully to satisfy these requirements. However, the possibility cannot be excluded that blood-group differentials may be a constituent part of the individuality differentials. It seems that a comparison of the ability of the erythrocytes to absorb various chemicals, and presumably also other physiological or pharmacological tests, indicate better the phylogenetic relationship of the species from which the red cells to be tested are derived, than the study of the relations between blood-group differentials and blood sera of various species of animals. As we have stated in a preceding chapter, various tissue and organ differentials, whether their significance is due to structural, biochemical or functional con-

subsequently employed for similar experiments by Uhlenhuth and his collaborators. They introduced a refinement in the precipitin test by obtaining the precipitins from animals belonging to one of the species whose blood was to be tested or from a related species. For this purpose, cross-immunization was used between two species, the relationship of which was to be studied by the precipitin test, each of the two species being injected with the blood serum of the other species. This method eliminated the coarser reactions which led to the production of less specific precipitins in farther distantly related animals which had been immunized against blood serum. Thus, by immunizing rabbits with hare serum Uhlenhuth could obtain specific precipitins for hare blood, although it was not possible to distinguish between individual rabbits. Similarly, through a slight modification of the same method, Black succeeded in differentiating Negro chickens from Italian chickens, individuals belonging to a third chicken species being immunized against Negro and Italian chickens. Subsequently, further refinements were introduced by Boyden, Hektoen, Wolfe, and Wilhelmi. The specificity of the precipitin test was enhanced by limiting the amount of protein injected into animals to be immunized, by dilution of the anti-sera, by using reciprocal tests and quantitative methods of determination, by removing the lipids from the antigens and in addition also by employing a nephelometric method of measurement. In some instances it was possible to distinguish between the serum proteins of such closely related animal species as ox, sheep and goat, and in some cases even between the proteins of buffalo and ox serum. With these improved methods it was also possible to differentiate between nearly related species of birds and of reptiles, classes of animals which had not been found very suitable in the experiments of Nuttall. By considering also the results of cross-reactions and of absorption or exhaustion tests of immune sera against egg albumins of various species of birds, differentiation between these species was possible (Hooker and Boyd, Landsteiner and van der Scheer). In using these precautions, the ring-test for the detection of precipitates permits not only the determination of relationships, but also the degrees of relationship between various species.

Still, it was found impossible to distinguish in every case by the ordinary precipitin method between man and anthropoid apes, and between horse and donkey. On the other hand, certain differences were more readily found between man and lower monkeys, and the Old World monkeys were shown to be more nearly related to man than the New World monkeys. It was also possible to distinguish between rat and mouse, and in recent experiments Landsteiner and Levine observed, in one case, that by the use of Uhlenhuth's method precipitins could be produced which differentiate the blood sera of man and chimpanzee. These investigators immunized a chimpanzee with human serum proteins and thus obtained an immune serum which precipitated only human serum but not chimpanzee serum. However, this differentiation could be much better accomplished by applying the Uhlenhuth method to the production of hemagglutinins. Furthermore, Hicks and Little traced, by means of the precipitin test, the relationship and origin of different species

Chapter 2

The Demonstration of Species Differentials by Serological Methods

AT THE END of the last and in the beginning of this century, when our knowledge of experimental immunity began to develop and it was found that immune bodies could be produced not only against bacteria but also against cells, which are normal constituents of the body of higher animals, and against proteins, such as those of the blood, the problem arose more definitely as to the chemical basis for the differences and the relationships between various animal species and as to the possibility of approaching this problem by the methods of immunology. It was important to know whether the relationship between different species and classes of animals, which so far had been studied mainly by the morphological methods of comparative anatomy and embryology, could be measured also by serological methods and whether the results obtained by these two methods agreed with each other. The chemical constitution of the cells and proteins serving as antigens, and the antibodies produced by the injection of these antigens into other animals should then correspond to the systematic relationship of the various species and they should show similar gradations.

Friedenthal in 1900 first studied the relationship of animal species by testing the compatibility between the transfused blood of a foreign species and the blood of the host species. Hemoglobinuria resulting from hemolysis of the strange blood corpuscles would signify incompatibility between the blood sera and the red blood cells of the two species. He also found in in vitro tests that only the erythrocytes of anthropoid apes resist solution by human sera, while the blood corpuscles of lower monkeys are dissolved. In these investigations the relation between preformed constituents of sera and erythrocytes was used as a test, rather than the reactions between an antigen and the immune substances resulting from injection of the antigens into a foreign species. Gruenbaum, in 1902, first used the precipitin test in analyzing relationships between species. This method depends upon the production, in an animal injected with blood serum from another species, of substances (precipitins) which have the power to precipitate specifically certain constituents of the serum used for injection. Neither Gruenbaum nor subsequent investigators were able to differentiate between man and anthropoid apes in this way. Two years later, Nuttall published the results of very extensive systematic studies, in which by means of precipitins he tested the relationship of many species, not only of vertebrates but also of invertebrates. In general, his findings confirmed the conclusions of zoologists as to the phylogenetic relationship of animals, which were based on morphological criteria. This method was

growing parts of plants, in which storage of proteins, and possibly also of other substances, is as yet less prominent than in fully developed parts, and especially less prominent than in seeds. Before using material from growing parts as antigen he freed it from its fatty constituents through extraction with alcohol and ether, and this procedure greatly increased the specificity of the reaction, which, as in the case of animal serum reactions, depends primarily upon the proteins contained in the antigen solutions. Mez assumed that the increase in specificity of the reaction, caused by previous absorption of the lipids from the tissue furnishing the antigen, is due to the lack of species-specificity of these lipid substances. By means of this method, Mez attempted to trace the development of plants from bacteria to algae and mosses, and from these to the higher organisms. More recently, Steinecke has extended these investigations. According to Boyden, these phytoserological studies have yielded data of crudely quantitative nature which support the concepts of plant phylogeny advanced already on the basis of morphological studies. In a similar way Wilhelmi found that the previous extraction of lipids from the antigens increased the value of the precipitin reaction as a method for determining phylogenetic relationships of helminths. After the lipids had been removed, the proteins acted as potent species-specific antigens, whereas the presence of lipids interfered with this reaction, because these substances by combining with a protein could function as haptens, which are less effectively species-specific and may be organ-specific.

In general, we may then conclude that substances of protein character, which differ in their constitution in different, not too nearly related species, may serve as antigens and lead to the production of precipitins which react specifically with the antigen by the formation of precipitates. If we compare the interactions of different antigens with the same immune serum, we notice that the strength of these reactions indicates the graded relationship of these antigens and of the animals from which they were obtained. By the introduction of certain refinements in the methods used, distinction may be made in this way also between more nearly related species, although as a general rule it is possible by the ordinary precipitin methods to distinguish only between species belonging to different orders, and it may be difficult to establish fine gradations even between different orders. In principle, then, these antigens behave like the coarser types of organismal differentials.

Soon after the precipitin test had been introduced as a serological test for phylogenetic relationship, Marshall, in the laboratory of Ehrlich, used for the same purpose, hemolysis, the solution of red corpuscles by means of preformed or of immune sera. Thus he found a close relationship between the antigens present in the erythrocytes of man and of Macacus monkeys. However, certain differences were observed in the hemolyzing power of active monkey serum for human and monkey erythrocytes, respectively, and differences between these two kinds of blood were established also by absorption tests, in which different antibodies present in the same immune serum could be separated from each other by specific absorption with erythrocytes from different species, to which these antibody fractions had specific affinities.

of mice. By immunizing rabbits, they believe that they succeeded in differentiating between Mus musculus and Mus bactrianus; however, Mus musculus could not be differentiated from Mus farvensis and Mus bactrianus behaved like the Japanese waltzing mouse. These investigators concluded, therefore, that Mus musculus is closely related to Mus farvensis, and Mus bactrianus to the Japanese waltzing mouse. But, as Boyden remarks, this serological differentiation of two so nearly related species is unusual and needs confirmation. By reducing very much the quantities of antigen used for immunization Wolfe was able to distinguish, by means of the precipitin reaction, between gray squirrel and red squirrel, and also between ox and sheep, but not between goat and sheep.

By means of reciprocal immunization Boyden found the dog to be more closely related to the pig than to the horse; still further distant from dog were beef and sheep; beef was nearest related to sheep, then followed horse and pig and dog. However, Boyden is careful to state that these tests merely indicate the relationship of these animals as they are constituted at present, and that they do not necessarily correspond exactly to the phylogenetic evolution of these species. Greater are the difficulties of distinguishing, by means of the precipitin reactions, species among reptiles and amphibia, if rabbits are used for immunization, although to some extent this, too, can be accomplished. This method, supplemented by absorption tests, made it also possible to differentiate between the hemocyanins present in the blood of various arthropods. Within a certain range there existed a relation between the strength of the precipitin reaction and the phylogenetic nearness or distance of the species used.

Different species of birds could be especially well differentiated serologically (Defalco), more readily than mammals, amphibians or fishes; it is therefore believed that birds represent a very homogeneous group. Blood serum, crystallized egg albumin and lens of the eye, or hemoglobin, when used as antigens, gave essentially similar results, except that in serum several proteins were present, which introduced certain complications, while egg albumin and hemoglobin represented essentially single proteins. Among invertebrates sharp differentiations could be made between species belonging to different genera as well as between others which belonged to the same genus. On the strength of his tests of helminth species, Wilhelmi believed that it was possible to define quantitatively the dilution of the antigen in the precipitin reaction which was characteristic of species differences. The results obtained with the precipitin reaction were also in conformity with the conclusion that echinoderms, and especially holothurians, were more closely related to prochordates than were annelids. In general, then, serological tests confirmed and made more secure earlier conclusions based mainly on structural studies.

The relationships of plants have been studied very extensively, by Mez and others, by means of precipitin tests, and they have thus traced the phylogenetic evolution of the vegetable kingdom. In contrast to the experiments in animals, where usually bodyfluids or some of their constituents were used as antigens, Mez employed for his determination, extracts of young,

pigs depended upon the chemical relationship between the substances used for sensitization and for reinjection, irrespective of the species of the plant from which the substances had been obtained. Thus, for instance, gliadin and hordein, although they occur in seeds of different species, could not be differentiated by means of anaphylaxis, because their chemical structure was similar. Corresponding results were obtained with other substances resembling albumoses in their reactions, which likewise were isolated from seeds; however, these substances were not, in all probability, split products of proteins, because real albumoses or peptones seem to lose their power to sensitize the guinea pig. With these materials from seeds, bcause of their solubility in water, anaphylactic reactions could be obtained much more readily than with the above mentioned alcohol soluble substances. The tests indicated that these albumose-like substances are quite distinct immunologically from the alcohol soluble substances, although both occur in the same kinds of seeds. A relative overlapping of reactions in experiments was apparently due to impurities, it being impossible to separate completely the first and the second type of substances.

Wells and Osborne concluded, then, that the specificity in the anaphylactic reaction depends primarily not on the biologic origin, but on the chemical constitution of the substances used for sensitization and the production of shock. But, the chemical constitution furnishes the basis for the biological specificity, and biological specificity depends upon the constitution of tissue constituents, and there should therefore be expected a correlation between the chemical constitution of plant proteins and the systematic position of the plants in which these substances originated. When, in the case of these plants substance-specificity was prominent, whereas species-specificity was not manifest, it may be assumed that besides the biologically important seed proteins, which could not be differentiated, there were other chemically distinct substances present in the embryo proper, which were not indicative of organismal differentials. In experiments with proteoses there was a slight interaction between those of pea and soy bean, two nearly related substances, and in more recent investigations Lewis and Wells found more definite evidence of a correlation between chemical constitution and systematic relationship.

By means of various anaphylactic methods, such as the uterus strip method of Dale, the bronchospasm method and the production of shock, as well as by the use of the complement fixation tests, these investigators observed that the alcohol soluble proteins from certain cereal grains can be separated into a wheat group and a corn group. The various proteins of the wheat group could not be differentiated from one another by immunological methods, nor could the members of the corn group be thus distinguished. On the other hand, there were sharp distinctions and a lack of cross reactions between two proteins which belonged to different groups; analogous proteins in related species behaved immunologically in the same way, but they were distinct from the proteins of further removed species. These results agree with the chemical analysis of these substances by Gortner and Hoffman, which showed the great chemical resemblance of the analogous alcohol soluble substances

More recently, Landsteiner and Miller were able to differentiate between the blood of man, of chimpanzee and orang-utan by means of immune hemagglutinins, which they produced in rabbits, against these various types of erythrocytes, but it was necessary first to remove, by specific absorption, the non-specific agglutinins which these species had in common. These investigators found that the differences between the blood of man and chimpanzee or orang-utan are less marked than those between these species and the lower monkeys. As mentioned above, Landsteiner and Levine furthermore succeeded in obtaining hemagglutinins which agglutinated only human but not chimpanzee erythrocytes, by injecting a chimpanzee with human erythrocytes, in this manner applying Uhlenhuth's method to the hemagglutinin test. In still another and more simple way they were able to differentiate between human and chimpanzee blood by the use of the preformed heteroagglutinins which occur in ox serum. After absorption with chimpanzee erythrocytes, ox serum still agglutinated human corpuscles very strongly and, conversely, after absorption with human corpuscles it was still active towards chimpanzee blood.

Certain additional methods were employed for the purpose of grading the relationship of antigens derived from different species. Thus alcohol extracts of various types of blood corpuscles in combination with a foreign serum, the protein of which served as carrier, could be used as antigens for the production of hemolysins. When these alcohol soluble, partial antigens were acted upon by the specific, heat-inactivated hemolytic immune sera, a flocculation and also a fixation of complement occurred, which were specific. Specific complement fixation has been employed for the testing of graded relationships of antigens by various investigators. Many years ago, Bruck believed that it was possible to demonstrate, through complement fixation, differences even between the blood of different human races, such as European, Malay, Arab and Chinese. However, this observation could not be confirmed by Marsball and Teague, nor by Fitzgerald; nor were Landsteiner and Miller able by serological methods to demonstrate differences between the blood cells of the white race and the Negro.

A further test for the specificity of antigens, especially of haptens which are non-protein components of antigens, was introduced by Landsteiner. Such haptens, which when injected alone into an animal belonging to a foreign species do not call forth the production of antibodies, do so if they are combined with a foreign protein acting as the carrier of the specific substance. But even without the aid of a carrier they may be able to inhibit in a specific manner the reaction between the antigen and the specific immune serum, irrespective of whether this reaction consists in precipitation, hemolysis, or hemagglutination.

Also, anaphylactic reactions have been used, especially by Wells and Osborne, for the testing of the organismal specificity of certain substances. These investigators worked with alcohol soluble proteins from various seeds, such as gliadin from wheat and rye, hordein from barley, zein from maize. First they showed that the occurrence of anaphylactic shock in sensitized guinea

tive (Lancefield). Also, specific nucleo-proteins isolated from streptococci could readily serve as sensitizers and likewise induce shock.

In general, it seems then that the specificity of the anaphylactic reactions is of about the same order as that of the ordinary precipitin and complement fixation tests. There is no indication that by means of the anaphylactic reaction it may be possible to differentiate between individuality differentials, although this reaction may very well serve for the demonstration of species differentials and of chemical substance-specificity.

Wells and Osborne were able to find in every case in which two substances were specific, as far as the anaphylactic test indicated such specificity, a definite chemical, in contrast to a mere stereoisomeric constitutional difference between these two substances; and this was true not only of the alcohol soluble plant proteins, but also of five substances derived from ovomucoid. From former data it might have been expected that also stereoisomeric differences between substances might give rise to specific serological reactions, and, as we shall see later, Landsteiner subsequently succeeded in demonstrating effective stereoisomeric differences in haptens by means of the precipitin reaction.

There still remains one point to be discussed. We have noticed that in the experiments of Wells and Osborne, and in those of Lewis and Wells, the specificity of the reactions was either absolute, one substance sensitizing exclusively against the substance used for the production of anaphylactic shock, or the reaction did not make possible the distinction between analogous substances from related plants. The quantitative gradations in the reactions corresponding to the gradations in phylogenetic relationship seemed to be lacking entirely in the earlier experiments, although there was an indication of such gradations observed in the later work. Wells, Osborne and Lewis used in their experiments purified substances rather than mixtures of substances as they occur in ordinary extracts from blood or organs. Their findings might suggest that the gradations which are so commonly observed in the case of immune reactions, result from the use of mixtures of antigens as they are present in the tissues and bodyfluids of organisms; from this point of view the graded relationships of different species would depend upon differently constituted, quantitatively graded mixtures of various substances which are characteristic of these species, and not on gradations in the structure of a complex protein or on a combination of a specific hapten of a non-protein nature, which differs in a graded way in different species, with the same or a similar protein serving as carrier. However, this conclusion would not be in agreement with some other well established facts. Thus we may recall the immunological differences between the whites of chicken and duck eggs, as shown in the anaphylaxis experiments of Dakin and Dale. Such differences are graded, although these investigators used crystalline substances in their experiments; in this case, therefore, the gradation in the reactions must have depended in all probability on graded differences in the chemical structure of single substances.

We have seen that by means of immunization it is possible to demonstrate

in the various species of the wheat group and their differences from those belonging to the corn group. However, Gortner also refers to differences in the number of chromosome sets which exist between different members of the wheat group and to corresponding differences in hybridizability between these species. It seems, then, that here cytological differentiations and the mode of interaction of spermatozoa and ova of various species during fertilization are much finer tests for the constitution of organismal differentials than the chemical analysis of the alcohol soluble proteins, or the serological methods, such as anaphylaxis or complement fixation.

In the case of some animal substances, Dakin and Dale, using the uterus strip method for the diagnosis of an anaphylactic state, were able to differentiate between the crystalline egg albumens of hen and duck. The specificity of this reaction could also be shown by means of specific desensitization. In more recent years, Landsteiner's demonstration that certain antigens are complex and consist of a combination of non-protein hapten and a foreign protein which acts as a carrier, was followed by attempted immunization against these complex antigens, and in this connection use was made also of anaphylaxis as a test for the specificity of various antigens and of the relative significance of these two component parts of the antigen.

In such experiments, in order to sensitize a guinea pig against a hapten, it was necessary to inject the latter in association with a foreign protein, the hapten alone not being able to cause sensitization. As to the means of producing shock in actively or passively sensitized guinea pigs through a second injection of the antigen, somewhat divergent results were obtained. Landsteiner could, in some cases, but not in all, produce anaphylactic shock in guinea pigs sensitized with an azodye-protein combination by injecting the azodye alone. It has even been maintained that it is possible to sensitize guinea pigs by injection of diazotized atoxyl alone and, moreover, to cause shock in animals thus sensitized by injection of the same substance; it was further assumed that under these conditions the injected animal's own serum may act as carrier. In guinea pigs sensitized against the polysaccharides, which are responsible for the type-specificity of pneumococci, it was necessary to inject the animal with the same kind of polysaccharide, in combination with a protein, for the production of shock. On the other hand, intradermal injection of the type-specific pneumococcus polysaccharide alone could bring about a specific inflammatory skin reaction.

However, a specific glucoside, which itself was not able to produce shock nor, correspondingly, to cause precipitation with antisera, inhibited in a specific manner the condition of shock, which otherwise would have resulted in sensitized guinea pigs, by the injection of the glucoside in association with a protein (Tillet, Avery and Goebel). Similarly, the hapten inhibited precipitation, which would ordinarily have resulted from the precipitin-antigen combination.

In the case of streptococci the specific carbohydrate, without the addition of foreign protein, could induce shock in passively sensitized guinea pigs, provided the immune serum used for passive immunization had been very effec-

against the erythrocytes—and presumably also against other cells—of a certain species. Thus specificities, if they exist, may be obscured by the existence of a multiplicity of preformed antibodies which may interfere with one another. Accordingly, Landsteiner and Levine found, as stated above, that a serum after absorption with chimpanzee erythrocytes, acted much less on chimpanzee than on human blood, and the converse effect was seen after absorption with human blood cells. Also, in other instances when sera and cells of sheep, goat, fox and dog were used, it has been possible through absorption with erythrocytes, or in some cases, with other cells of a certain species, to remove from a serum, in a specific way, the agglutinins acting on the cells of this species, while leaving behind the agglutinins for the erythrocytes of another species. In some instances however, this absorption did not act in such a specific manner, but with the specific agglutinins for the cells of one species there were removed also those acting on the cells of a different species.

Furthermore, when once a definite threshold of strangeness has been reached, a finer differentiation among the strange species, as to the degree of toxicity of substances present in their sera or cells, will hardly be possible. This applies as far as preformed substances in sera and cells are concerned, but not in the case of immune sera. In a similar way, we found that in heterotransplantation the conditions existing in the host are so intensely injurious for the transplant that finer gradations in the degree of injuriousness of different species, in accordance with the systematic relationship between host and transplant, are very difficult or even impossible to accomplish.

As to the effects of the injection of foreign sera into the circulation of rabbits, we noticed that these animals succumb readily when a certain amount of the heterogenous serum has been injected with a given rapidity. But again, different factors may be responsible for such a fatal outcome in the case of sera from different species and this diversity of factors precludes a strict parallelism between the systematic relationship of the various species used and the toxicity of their sera. Thus, according to Strickler, Tuttle and Loeb, intravenous injection of dog serum kills the rabbit, essentially, by the hemolysis it produces in the blood vessels of the rabbit. The products of hemolysis cause coagulation of the blood in the living animal in the same way as in vitro. Accordingly, the pulmonary vessels and the vena cava become filled with blood clots and the animal dies from asphyxiation. If the formation of blood clots is prevented by injection of hirudin or heparin into a vein previous to the injection of the serum, the latter does not kill the rabbit. The heparin diminishes hemolysis as well as the coagulation of the blood (Rabinovitch). Beef serum, on the other hand, causes, in vitro as well as in vivo, agglutination of the erythrocytes; the clumps of red corpuscles formed occlude the pulmonary vessels and death results. Directly after the death of the animal these agglutination thrombi may be demonstrated by exerting pressure on the pulmonary vessels, which forces the thrombi out of the cut vessels (Rabinovitch). It is possible that in addition to these toxic effects of dog and beef serum, other factors are involved in some instances; thus Zinsser maintains

in the sera of the immunized organism the presence of immune substances which react specifically with the antigens used for their production, but which also react more weakly with analogous substances from related species in a graded manner, in accordance with the graded relationships of the various species. How do preformed agglutinins and hemolysins behave in this respect? Do they show the same degree of specificity as do the immune substances?

There are present in the sera preformed antibodies which react with group antigens in the erythrocytes. The question now arises whether there exists a specific adaptation between preformed antibodies in sera, on the one hand, and erythrocytes and other cells, on the other, in accordance with the systematic relationship of the organisms from which the sera and cells are derived. Such a specific adaptation would be comparable to that which obtains between constituents of the plasma and tissue coagulins, and which becomes manifest in the coagulation of the blood. We have already discussed the fact that a specifically graded relationship between blood sera and erythrocytes of various species, corresponding to the phylogenetic relationship, has not yet been demonstrated by means of hemagglutination. Marshall likewise found that normal heterogenous sera of goat, sheep, goose, or rabbit were equally hemolytic for human and Macacus blood. Landsteiner observed that normal hemagglutinins absorbed by certain erythrocytes and then dissociated from this combination by elution, are active with the red corpuscles from numerous near and distant species. Thus solutions of agglutinin obtained by washing rabbit erythrocytes, which had previously been agglutinated by beef serum, acted intensely with both rabbit and frog erythrocytes. Landsteiner concluded therefore that agglutination or failure of agglutination of erythrocytes by the normal serum of another species is almost independent of the systematic relationship of those species. As far as agglutination of erythrocytes is concerned, this lack of agreement is understandable, since within the same species corpuscles from different individuals differ from each other as regards their agglutinability by the sera of other individuals of the same species, and since not only agglutinins exist in the sera of various species which agglutinate human red corpuscles, but heterogenous sera in general may agglutinate corpuscles from other species, irrespective of systematic relationship. Likewise, the presence of Forssman differentials and of other antigens of a similar kind, which, as we have seen, do not conform to the laws of systematic relationship, may interfere with and prevent a parallelism between the reactions of sera on cells of a heterogenous nature and the systematic relationship of the various species, genera, orders and classes of animals from which the sera and cells are derived. Thus, according to Klopstock and Lehmann-Facius, the sera of species possessing Forssman antigens dissolve cells of various species, irrespective of whether they belong to the heterogenetic or the non-heterogenetic series; on the other hand, sera from non-heterogenetic species dissolve only cells from heterogenetic species. But in addition, another factor may interfere with the manifestation of a parallelism between the toxicity of heterogenous sera and the systematic relationship of two species. There seem to occur in the same serum multiple constituents, each one directed

largely negative results are due not so much to the lack of these species differentials in the respective tissues, as to the difficulty experienced in extracting them in a potent form, and there are indications that, by injecting organ suspensions into rabbits, species-specific antibodies may be obtained. In the preparation of antigens from invertebrates for the production of precipitins, it is customary to make extracts from the whole animal after it has been frozen, dehydrated and ground to a fine powder; however, this material contained tissues as well as bodyfluids.

As we have stated previously, heterotransplantation of various tissues and organs shows the presence of heterodifferentials, which are not limited to one kind of tissue but which are distributed throughout the body similarly to the individuality differentials. But the gradations in the results of transplantations are not as delicate and as definite in the case of species differentials as in the case of individuality differentials. In that part of the spectrum of relationships which represents the heterodifferentials, the analysis by means of serological methods is at least equal, and probably superior, to the analysis by means of transplantation. In the individuality differentials, on the other hand, we have to deal evidently with much more delicate and specific substances than in the species differentials, and here serological analysis is the less refined method. And yet, each species has its characteristic species differential. Is this species differential attached to a particular substance, which is the same in all organs of a species, or do different substances assume these functions in different organs? There is no doubt that different substances, present in different organs or tissues, may possess species differentials exhibiting the specific effects. The evidence so far points strongly to the conclusion that there is one chemical feature which characterizes a species and that this may be attached to various substances, which are thus the bearers of the species differential; and, as a rule, the demonstration of serological differences in the experiments discussed in this chapter depended upon the presence in the proteins of this species differential which served as an antigen. However, it seems that in some instances in which certain plant proteins were used for the sensitization of animal, tissue- or substance-specific material may have called forth sensitization and the subsequent anaphylactic reaction, and it is probable that also in animals a combination of tissue and organ differentials may to a certain extent, and with certain limitations, substitute for the real species differentials.

that goat serum may, in certain cases, kill rabbits by a mechanism comparable to that which is effective in anaphylaxis.

While these facts make understandable the lack of a strict quantitative correlation between the systematic relationship of animals and the action of heterogenous blood serum on cellular constituents, still, a specific adaptation between sera and the cellular elements does exist. This is evident from the fact that, as a rule, autogenous and homoiogenous blood sera are much more favorable for the preservation of the cellular constituents of the blood than are heterogenous sera. This is true also of man and of certain animal species in which blood groups occur, if the group agglutinins are first removed from the blood. There are indications that similar conditions hold good in the case of the blood of invertebrates as well. Thus we found the blood serum of Limulus on the whole more suited for the normal activities of experimental amoeboeyte tissue of Limulus, than the blood serum of decapode arthropods, such as the lobster. However, through previous heating of lobster serum the latter can be converted into a favorable medium. Furthermore, Limulus serum on being mixed with the serum of other Limuli remains clear, but after mixing it with the sera of various crustaceans, precipitates usually develop.

While thus, in general, no very strict quantitative parallelism can be demonstrated between the action of normal sera on heterogenous cells and the relationship of the organismal differentials of the species used, such a specific relationship is demonstrable in the case of immune sera, produced through injection of sera or cells into an animal possessing different organismal differentials. As a rule, the antigenic activity of the species differentials predominates under these conditions over that of other antigens contained in the cells.

We may then conclude that not only in the cells of an organism, but also in its blood serum, there are present species differentials which can function as antigens, and which, by means of experimentally produced immune substances, can readily be demonstrated; also, that these differentials show a gradation corresponding to the systematic relationship of the organisms. Moreover, we have found that the cells of an organism and its normal blood plasma contain species differentials which are mutually adapted to each other; but a graded relationship between the organismal differentials of the cells and bodyfluids belonging to different species and orders of animals, which would correspond to the systematic relationship of the animals, cannot as a general rule be demonstrated in the interaction between sera and cells.

In these investigations, which concern the differentiation of different species by serological methods in animals, substances and cells, which are constituents of the blood, have been used in most experiments. While in plants substances which are present in young tissues may also serve as species-specific antigens, it seems to have been difficult, so far, to extract from organs of higher animal species antigens which, after injection into other species, would give rise to the production of precipitins or of complement-fixing substances. Such antigens, if present at all, are found only in very small quantities and show only a slight degree of specificity. But, it may be suspected that these

which are obtained in the analysis of individuality differentials by means of transplantation, the reaction depending here not merely on the nature of the transplant, but on the relation between the individuality differential of the donor and of the host. While it was thus possible to produce substances which Ehrlich and Morgenroth called "isolysins"—but which would, perhaps, better be called "homoiolysins"—in no case did such a homoiohemolysin dissolve the erythrocytes of the animal which had produced that particular hemolysin. Autohemolysins did not develop under these conditions. However, in some goats it was not possible to elicit the production of homoiohemolysins in this way; here the injected homoiogenous red corpuscles behaved like autogenous cells. Another difficulty was that the antisera not only reacted with the red corpuscles of the goat which had furnished the antigen, but also with the erythrocytes of a number of other goats. These facts suggested to von Dungern and Hirschfeld, as well as to Witebsky, the interpretation that Ehrlich and Morgenroth had in reality not to deal with individual hemolysins, but with group isolysins corresponding to the group hemagglutinins, the occurrence of which in certain animal species had been demonstrated by von Dungern and Hirschfeld. Also, Zinsser assumed that while Ehrlich and Morgenroth had actually discovered individual differences between the red corpuscles of different goats, these individual differences were identical with blood-group differences.

As discussed previously, it is necessary to distinguish between at least four different kinds of substances: (1) The typical group differentials, which are represented in man by the agglutinogens A and B; (2) accessory blood-group differentials, such as M, N, P, Rh and H; (3) substances which allow the distinction of individuals, as for instance, individual scents, and also certain tissue or organ differentials, or combinations of the latter. While a combination of a sufficiently large number of accessory blood-group or organ differentials might permit the distinction between individuals, this would not make these individual differences necessarily identical with (4) the individuality differentials. It is difficult to determine whether Ehrlich and Morgenroth had to deal with substances enumerated under 2, 3, or 4.

However this may be, the desire of Ehrlich and Morgenroth to determine the possibility of the formation of "isohemolysins" suggested to them the use of a method which allowed a much finer differentiation between individuals than had been possible previously by means of serological methods, in particular, those in which the ordinary heterogenous immune sera were employed. These investigators anticipated the essential feature of the method of cross immunization, subsequently introduced by Uhlenhuth with a view of refining the precipitin test. Furthermore, the use of cells instead of blood proteins as antigens may have been a favorable factor which made possible the demonstration of individual differences by serological methods.

The fact that homoio (iso) hemolysins can be produced experimentally was subsequently confirmed by Ascoli and by various other investigators. But the most extensive and important studies concerning the demonstration of individual differences between the red blood corpuscles of different individuals,

Chapter 3

The Demonstration of Individuality Differentials by Serological Methods

In the preceding chapter we have discussed various investigations which tended to prove the existence of species, generic and class differentials by means of serological methods. We have seen that the precipitin test in general permits only the distinction of relatively far distant species, but certain refinements in technique may make it possible to distinguish also between more nearly related species. At an early period of these investigations, it was especially Hamburger who suggested that not only species differed in their chemical constitution, but that also individuals might differ. It was therefore natural that the attempt should be made to demonstrate differences between the proteins of different individuals by means of methods similar to those used for the demonsfration of species differentials. Weichardt seems to have been the first to make experiments of this kind. He believed that he was able to demonstrate individual differences in the degree of precipitation taking place on mixing the blood proteins of two individuals with their respective antisera, after previous saturation of the antiserum of one with the serum of the other individual. The sera then appeared to react more strongly with the individual antigen used for immunization. Weichardt used heterogenous immune bodies in his investigations and in the light of what we have since learned concerning the limitations of the precipitin test, it is very improbable that individuality differentials can be demonstrated by these means.

However, a few years previous to this work Ehrlich and Morgenroth, using a different technique, had actually shown the existence of individual differences between antigens, in experiments to which we have referred already. But it seems that these investigators were not primarily interested in the analysis of what we now would designate as individuality differentials; they wished, rather, to determine whether immune substances could be produced only against heterogenous substances, and whether a condition which Ehrlich had named "Horror autotoxicus" would prevent the formation of antibodies in an animal of the same species. They therefore injected massive doses of hemolyzed blood corpuscles of thirteen goats into other goats and obtained thirteen hemolysins for the blood corpuscles of the individuals which had served as donors of the antigens. A comparison of the effects of the different immune sera on the blood corpuscles of the various individuals showed that the sera were not all alike, but that each one behaved in a distinctive manner. It could furthermore be shown that the differences depended not only on the kind of corpuscles injected, but also on the animal which had produced the hemolysins. Thus two different goats, injected with the same goat blood, gave different hemolysins. This corresponds with the results

consider the possibility that in the corpuscles of the calf the antigens were quantitatively not yet as fully developed as in the corpuscles of the mother. Examination of a family of sheep, consisting of father, mother and three lambs, showed that the corpuscles of one lamb behaved in an almost identical manner with those of the mother, while the corpuscles of the other two lambs had the character of the father. Here, too, there is a lack of gradation, and there is again reason for assuming that under similar conditions transplantation would in all probability have revealed graded differences between the constitution of the cells of the various children and of father and mother. Another peculiarity in these experiments needs particular mention, namely, the importance of the race to which the individual cattle belonged. We should have expected the erythrocytes of a certain individual to resemble more the corpuscles of an individual belonging to the same strain than the corpuscles of an individual from a different strain, but this was apparently not the case. In transplantation experiments, on the other hand, the differences existing between different strains, such as white, yellow and piebald strains of rats, and also those between inbred strains of guinea pigs and mice, have a distinct effect on the reaction of the host against the grafts.

In more recent experiments Todd analyzed in a similar manner, individual relationships in fowl by means of immune hemagglutinins. Here, too, it was found that by absorption tests the red corpuscles of each individual animal could be distinguished from those of others. Some erythrocytes resembled each other more than others; but certain members of the family could not be distinguished from one another by this method. In these experiments polyvalent sera were used. If they were absorbed with the corpuscles of one individual, only immune substances directed against this individual and against some near relatives were thereby removed; but if the polyvalent serum was exhausted with the red corpuscles of several individuals, the number of immune substances removed was greater. Also, in this case we find a lack of a furthergoing gradation in the relationship of the various individuals. In analyzing the relationship between parents and children, Todd found, again, the corpuscles of some children behaving like those of the father, others like those of the mother; but in two instances the antigens of the children possessed components of both father and mother. If a polyvalent serum is absorbed with the erythrocytes of both parents successively, it has of course, lost also the agglutinins for the cells of the child completely. Theoretically, the red corpuscles of the child should have components of the parents to an unequal degree in the large majority of cases. This is found in transplantation experiments and they suggest that there does not, as a rule, exist an identity between the structure of the differentials of a child and of one of the parents.

An indication that it is perhaps the differentials which serve to distinguish the erythrocytes of various individuals, and that they also function as antigens in immunization, was obtained in experiments in which the production of agglutinins against the blood corpuscles of brothers and sisters was tested. In each instance, Todd injected a chicken witb the blood of a brother. Several

belonging to the same species, were carried out by Todd and White, and later by Todd alone..We have referred already to their work and we shall now again discuss these investigations. There can be little doubt that these latter investigators had to deal with individual and not with group differentials in their investigations. Todd and White, and Todd, in preparing immune serum against cattle plague, injected different cattle with the blood of other cattle. Out of one hundred and six cattle injected, seventy-six furnished active sera containing homoio (iso) hemolysins for normal cattle, which were active in combination with guinea pig complement. Each immune serum thus obtained was able to hemolyze the red corpuscles of certain other individual cattle, and this action differed from that of the immune serum obtained from another animal. Thus a particular serum was very hemolytic for some kinds of erythrocytes and only weakly hemolytic for others, and each serum acted in its own specific way on the various kinds of corpuscles. The order in which two different sera affected a series of corpuscles from different individuals was different in each case.

These individual variations between the antigens present in the corpuscles of different cattle were brought out still more strikingly in specific absorption experiments. If a certain serum was exhausted by the addition of the red corpuscles of an individual animal, it lost thereby not only the ability to hemolyze the kind of corpuscles which had been used for absorption, but also the erythrocytes of some other individuals; moreover, a gradation between the erythrocytes of different individuals according to genetic relationship, such as had been observed in transplantation experiments, did not apparently exist here; it seemed, rather, that "an all or nothing" law obtained. But if several immune sera were pooled, the absorption tests became more specific, in so far as now absorption with the corpuscles of one particular animal removed, primarily, the hemolysins of this individual, leaving the others as a rule intact. Still it might happen here also, that not only the hemolysins for those individuals whose corpuscles were used for specific absorption were removed, but also the hemolysins for some other individuals. It appears that if the relationship between two individuals did not exceed a certain degree of remoteness, their erythrocytes behaved alike in the absorption test, a finer quantitative gradation in the intensity of the reaction, such as can readily be accomplished by means of transplantation, being impossible in this case. However, in an indirect manner, by comparing the behavior of the corpuscles of various individuals to different immune sera it might perhaps have been possible to establish the mutual relationship of the corpuscles of the various individual cattle, at least in an approximate manner.

The same lack of gradation was also apparent in the analysis of the relationships of the members of certain families by means of the hemolysis test. Thus the blood corpuscles of a cow and her calf were compared as to their specific ability to absorb the individual hemolysins. It was found that absorption with the corpuscles of the mother removed also the hemolysins for the calf, but absorption with the corpuscles of the calf left the hemolysins of the mother intact, while removing those for its own corpuscles. In this case we have to

on the other hand, the character of various tissues and organs of one individual is contrasted with that in another individual; this is possible because the individuality differential is not merely an attribute of one particular kind of cells, such as the erythrocytes, but is present in the various tissues and organs of an individual.

We find, in general, in the serological tests a lack of those fine gradations between intensities of reaction, which correspond to the degrees of relationship of the partners, observed in cases of transplantation. In transplantation experiments the cells and tissues transferred to a strange environment set in motion finely graded cellular mechanisms of attack in the host and the transplants are also acted upon by the graded injurious actions of the host bodyfluids. In the experiments of Ehrlich and Morgenroth, as well as in those of Todd, the antigens of the red corpuscles initiated the production of immune hemolysins or immune agglutinins. On the whole, these latter reactions resembled either autogenous or fully developed homoiogenous reactions, although in the hemolysis tests certain gradations in the intensity of hemolysis were found in different combinations of corpuscles and immune sera in some instances, and such gradations were apparently in accordance with the relationship between the animal whose blood corpuscles were tested and the animal serving as the immune-body producer. Is this difference between the reactions following transplantation and the effects of immune sera due to a difference in the differentials or antigens which participate in these two tests? Presumably it depends largely on the more finely graded reactions exhibited by living tissues, as compared with the in vitro reactions between antigen and antibody.

The antigens present in the erythrocytes are substances which can be partly or wholly neutralized or removed through absorption with the corresponding antisera. When a certain degree of relationship exists between the donors of the erythrocytes and the various animals to be injected with these cells, the correspondence between the immune bodies and the antigens may be sufficiently great to make possible the complete removal of these immune substances by the erythrocytes of the donor. These differences between antigens may conceivably depend upon chemical groups forming part of one complex substance, or perhaps we may have to deal with distinct substances. These antigens, which ultimately are derived from genes situated in the nucleus, are themselves situated outside the nucleus; at least this is the case in the nonnucleated erythrocytes.

We have seen that the individuality differentials are preformed and there is reason for assuming that they elicit homoioreactions in transplantation directly, at least to a large extent, and that these reactions do not primarily depend upon the formation of immune bodies, although secondarily, immune reactions may occur. Similarly, the solution of the foreign blood corpuscles, after intravenous injection into homoiogenous hosts, depends primarily upon the incompatibility between the strange individuality differentials of the erythrocytes and the bodyfluids of the host, and the formation of immune substances is a process caused by this primary incompatibility. While it has

pairs of this kind were tested in this manner; in two of them antibodies were readily produced, whereas in two other pairs immunization was obtained with greater difficulty. In the latter cases, in testing the agglutinating power of the sera for the blood corpuscles of the two partners, almost no distinction was found; correspondingly, each had very weak antigenic potency in the partner. On the other hand, the partners in the first two pairs could be readily differentiated from each other and, accordingly, distinct effects were obtained. The agglutinating sera resulting from immunization with the erythrocytes of brothers were highly specific and acted only on corpuscles of birds very similar to those whose erythrocytes had been used for injection; but towards these cells they appeared to be as active as the sera prepared by injection of non-related fowls; there was again a lack of gradation.

In chickens, individual differences were found also by Landsteiner and Miller, who immunized rabbits with chicken blood. The immune agglutinins in the rabbit serum could be absorbed with the red blood corpuscles from a certain chicken. The remaining rabbit serum was still able to agglutinate the erythrocytes from other chickens but not those from the animal used for absorption; only in two pairs of chickens were the agglutinins found identical in these tests. Similarly in natural ox serum multiple substances seem to exist, which are able to agglutinate the red corpuscles of individual chickens. Through absorption with the erythrocytes of a chicken these substances could be specifically removed. Ox serum thus treated no longer agglutinated the chicken corpuscles used for absorption, although it had retained the ability to agglutinate the corpuscles from other chickens (Landsteiner and Levine). Likewise, by injecting chimpanzees with human erythrocytes, Landsteiner and Levine were able to find some differences between individual human red corpuscles. However, in the case of turkey and guinea fowl blood, individual differences could not be established by these means.

In the experiments of Todd, and in similar investigations of others, as well as in the earlier experiments of Ehrlich and Morgenroth, the question arose as to whether we have not to deal with group antigens rather than with individual antigens. Thomoff, in experiments on the formation of homoiohemolysins or homoioagglutinins in horses, suggested that reactions occur only if the donor of the antigen and the producer of the immune substances belong to different blood groups. However, even in these experiments the hemolysins or agglutinins were not primarily antibodies against the group antigens of horses, but they were individual agglutinins and hemolysins, although secondarily the group differentials may have entered as a factor in these reactions. Similarly, it may be possible that also in Todd's experiments the group differentials may have played a secondary role, but essentially these experiments concern differentials distinguishing individuals.

In comparing these serological tests for individuality with the analysis of the individuality differential by means of transplantation, we see that in the former use is made of the antigen of one type of cell only, the erythrocytes, and the conclusions likewise relate merely to the differences between the antigens in various kinds of red blood cells. In transplantation experiments,

stances through immunization with homoiogenous erythrocytes, but that a hemolytic immune serum thus obtained does not hemolyze the red corpuscles of the individual in which the immune serum developed. It is therefore impossible to elicit a reaction against autogenous cells. Similarly, Ehrlich and Morgenroth have shown that antihemolysins cannot be made to appear by injecting isohemolysins into a goat in which the hemolysins had originated. Likewise in the case of tumor immunity, we have seen that an active immunity against the growth of a transplanted tumor will not result from inoculating an animal with pieces of its own organs.

However, it has been held that in certain cases autogenous antibodies may actually be formed, but not all the authors distinguish sharply between substances and reactions of an autogenous and homoiogenous nature, and it is thus difficult to determine whether we have actually to deal with autogenous rather than with homoiogenous reactions. To mention some examples: According to Guyer, an injury to the eye-lens of a rabbit elicits in this animal the production of antibodies against lens tissue which enters the blood serum; these antibodies can be demonstrated by the formation of a precipitate on mixing the serum of the animal which has been injured with homoiogenous lens substance. Similarly, according to Henshaw, antibodies develop against autogenous antigens after exposure of the skin to ultraviolet rays; either by means of anaphylactic shock or by the precipitin reaction with corresponding homoiogenous skin material, the development of antibodies could apparently be demonstrated. In these cases we may perhaps have to deal with tissues, which, as a result of injury, had undergone chemical changes of a kind which seem to have made possible the formation of auto-antibodies. This applies also to the experiment of Letterer, who, by injection of autogenous venous blood, sensitized a guinea pig against its own blood, which caused a reaction when injected parenterally. Apparently the normal circulating blood does not cause such a sensitization.

In the case of paroxysmal hemoglobinuria, pathological changes of a specific kind have evidently taken place in the blood of certain individuals. As a consequence of these changes, it seems that autohemolysins develop, and the union between erythrocytes and autohemolysin which follows depends on an exposure of the erythrocytes to a low temperature. But it is not certain that in this instance there is actually involved an antibody formation against autogenous cells. Certain non-specific procedures, such as injection of boiled milk, apparently intensify the autohemagglutination in some rabbits, but injection of erythrocytes, normal or injured, does not have a corresponding effect.

In general, it may therefore be assumed that the body does not react against its own normal cells with the production of immune substances, while it is able to do so against homoiogenous substances. However, we cannot exclude the possibility that if a body is injected with its own injured cells, in certain cases a reaction may be elicited, but that such a reaction is less strong than one produced by means of injections of homoiogenous, or better still, of heterogenous material; furthermore, it is possible that substances of an autogenous

been possible to produce homoiogenous immune hemolysins or immune hemagglutinins in goats, cattle, chickens, and perhaps in some other species, according to Todd it is not possible to obtain a corresponding formation of homoiogenous immune bodies in guinea pigs and rats; but these are exactly the species which, above all, have been used in transplantation experiments and this is an additional reason for assuming that in the case of homoiogenous transplantation we have primarily to deal with incompatibilities between host and transplant, and with primary reactions of attack or defense, and only secondarily with immune reactions.

As we have pointed out above, in those experiments in which individual differences between cells could be demonstrated by serological methods, homoio-immunization was used in the preparation of the immune sera in the majority of the experiments, and in all cases erythrocytes or their derivatives served as antigens. There is, however, at least one instance on record in which apparently hetero-immunization with another type of cells also led to the demonstration of individual differences. According to Dervieux, by means of repeated injections of fresh human sperm into rabbits, antisperm precipitins can be produced, which have the strongest effect on the individual sperm of the donor; here they are effective in the greatest dilution. Dervieux found, furthermore, that the immune serum thus obtained had a stronger precipitating power for strange human sera than immune sera produced by injection of human serum; also, it allowed the distinction between individuals, and even between men and women. However, the spermatic fluid used by Dervieux, and also in the subsequent investigations of Süssman, contained not only spermatozoa but also other material, among which were admixed proteins. Therefore, the immune substances elicited by the injection of sperm may readily have been directed against these admixtures rather than against the spermatozoa as such. These experiments were apparently confirmed by Süssman as far as the individual specificity of the sperm antigens, but not of the blood protein antigens, is concerned. But it seems that Süssman carried out only a small number of experiments and not all of these were confirmatory of Dervieux's conclusions. It will therefore be necessary to wait for a confirmation of Dervieux's investigations before his results can be fully accepted.

More recently, Zangemeister indicated another method by means of which he thought it possible to differentiate between the blood sera even of nearly related individuals. He assumed that following fertilization of the ovum by a spermatozoon and the subsequent formation of the embryo, there develop, as the result of the entrance of sperm material into the blood serum of the mother and of the child, substances which cause a change in the state of dispersion of the serum proteins if the serum of the father is mixed with the serum of the mother, or if the serum of one of the parents is mixed with that of the child. This change in the state of the serum proteins was thought to indicate the relationship between the individuals whose sera were allowed to act on each other. However, these experiments could not be confirmed by Lattes.

We may then conclude that it is possible to produce specific immune sub-

Chapter 4

The Organismal Differentials of Hybrids Between Nearly Related Species

In all conditions which we have studied so far, in which serological methods have been used for the analysis of relationship, we were able to compare the results with those obtained by means of transplantation. There is, however, one type of relationship in which such a comparison between these two methods of investigation is not feasible at present. The mutual relations of hybrids between nearly related species, as well as the relations between the hybrids themselves and their parents, have been analyzed by serological methods, but only in a rudimentary manner by transplantation methods; the number of experiments representing the latter, made by Schultz, is very small. As Jacques Loeb has pointed out, a comparison of the species characteristics of an F_1 hybrid with those of the father and mother species should give an indication as to whether the rules of Mendelian heredity apply to the transmission of species characters, or whether hereditary transmission in this case takes place through the cytoplasm of the egg. If it takes place through the cytoplasm, the hybrid should resemble the mother species, but not the father species. Jacques Loeb suggested that it might also be possible to determine this question by comparing the characteristics of hemoglobin crystals of mule with those of horse and donkey. But the measurements of Brown showed that the mule crystals were outside the range of figures found for horse as well as for donkey, although they were somewhat more nearly related to those of the donkey. Thus crystallography did not help in solving this problem.

I. In order to test the relationship between horse, donkey, and the hybrid between these two species, the mule, use was made by Walsh of the presence of preformed hemolysins and hemagglutinins in these three types of sera acting on the various kinds of erythrocytes, while Landsteiner employed for this purpose immune agglutinins. The findings of Walsh were as follows: (a) Horse serum does not hemolyze horse, donkey or mule erythrocytes. Donkey serum hemolyzes both horse and mule erythrocytes in a large percentage of cases. Mule serum does not hemolyze horse or mule erythrocytes. (b) Horse serum agglutinates neither horse nor mule erythrocytes. Donkey serum agglutinates the erythrocytes of the horse in a high percentage of cases, but does not agglutinate the erythrocytes of the mule. Mule serum does not agglutinate horse or mule erythrocytes. From these observations it may be concluded that mule serum behaves like horse serum, and mule corpuscles behave like horse corpuscles rather than like donkey corpuscles, except as far as the agglutinating action of donkey serum is concerned. However, according

nature, not normally circulating in the bodyfluids, may call forth the production of immune substances if they gain access to the circulation.

It may then be concluded that in the erythrocytes of several species the presence of differentials of an individual character has been established by means of immune sera, but it has not yet been definitely proved that these differentials are identical with the individuality differentials. Even in some preformed sera, such as ox serum, multiple agglutinins seem to exist, which can be specifically absorbed by the erythrocytes of certain individuals. This fact may be interpreted as indicating that complexities in the structure of blood proteins exist, which have not yet been amenable to a purely chemical analysis.

were relatively slight and these investigators preferred therefore the use of immune agglutinins, which they produced in rabbits by injection of the different types of red corpuscles. They tested the immune sera thus obtained as to their action on the erythrocytes of horse, donkey and mule, either directly or after previous absorption of the immune sera by the various types of erythrocytes. All of these tests showed that the mule erythrocytes contained agglutinogens of both horse and donkey. However, in the mule red corpuscles, either not all of the donkey and horse agglutinogens were present, or they were present in smaller amounts. As a rule, the immune serum prepared in rabbits by injection of mule erythrocytes behaved more like anti-horse-corpuscle immune serum than like anti-donkey immune serum, notwithstanding the fact that the mule red corpuscles contain both kinds of agglutinogens. Horse as well as mule erythrocytes could bind the immune agglutinins from immune serum against mule corpuscles, including also the agglutinins which act on donkey erythrocytes; but after a previous absorption of such sera by donkey red corpuscles, a high agglutinin titer for horse and mule erythrocytes still remained in the serum, while the donkey agglutinins had been removed. It may therefore be concluded that as far as the production of immune sera can be used as an indicator, the horse agglutinogens predominate in the red corpuscles of the mule.

II. More recently Landsteiner studied, by means of immune hemolysins and immune hemagglutinins, the relations of the blood of hybrids between the domestic guinea pig (Cavia rufescens) and the wild Brazilian guinea pig (Cavia porcellus) to the blood of the parent species. In this case Landsteiner made use of homoio-immunization in order to eliminate the complication which the strangeness between the donor of the blood and the animal to be immunized would have introduced. Normally, no hemolysins or hemagglutinins are present in the blood serum of guinea pigs belonging to one species or race for the erythrocytes of the other. But by injecting the blood corpuscles of Cavia porcellus into Cavia rufescens it is possible to produce immune hemolysins and immune agglutinins in the serum of the latter, which act on the erythrocytes of Cavia porcellus, but not, as a rule, on the red corpuscles of Cavia rufescens. It was found that the red corpuscles of the hybrid behaved in an intermediate way; they contained characteristics of both parents. This result corresponds to our findings in the analysis of hybrids between different families in rats and in guinea pigs by means of transplantation.

III. Irwin tested, by means of immune hemagglutinins, the relations between the domestic Ring dove (Streptopelia risoria), the Asiatic Pearlneck (Spilopelia chinensis), and the hybrids between these two genera. Rabbits were immunized separately against the red corpuscles of the two parents and of the hybrid and use was made of the absorption of the specific antibodies by the various kinds of erythrocytes. It was found that the agglutinogens of both parents were present in the erythrocytes of the hybrid, and that also in the immune serum against the corpuscles of the hybrid, agglutinins against the corpuscles of both parents could be demonstrated. However, the erythro-

to Landsteiner, donkey serum does agglutinate mule corpuscles, although not to the same degree as horse corpuscles. Agglutination tests indicate, therefore, that mule blood behaves essentially like horse blood, but in some respects it shows the influence of the donkey. It seems, then, that in this species hybrid the characteristics of the female parent predominate, suggesting the possibility that we may have to deal at least with a partial cytoplasmic inheritance.

Landsteiner wished to determine whether the blood groups which are present in the horse, and lacking in the donkey, are transmitted to the mule. Von Dungern and Hirschfeld had previously observed that the isoagglutinins of the horse are transmitted to the mule. In the donkey, isoagglutinins are not demonstrable. By means of immune sera and absorption tests Landsteiner and Van der Scheer found in the erythrocytes of the horse more than three kinds of isoagglutinable substances. The mule inherits from the horse isoagglutinable groups, but not all the blood groups are equally transmitted from horse to mule. The blood of the majority of mules belongs to the class in which the serum contains agglutinins, and the corpuscles are not, or only slightly, agglutinable. Not as many mules contain the isoagglutinable substances as do horses; this agrees with the fact that donkey blood contains no blood groups with isoagglutinable erythrocytes. There is therefore, again, an influence of the genetic constitution of the donkey noticeable in the mule so far as the inheritance of the blood groups is concerned. But horse agglutinogens are transmitted to mule corpuscles, which lead to heteroagglutination of mule corpuscles by donkey serum. Donkey serum behaves therefore in the same way to a certain group of horse and mule corpuscles; however, it does not cause the agglutination of mule corpuscles to the same degree as it does that of horse corpuscles, and there are more individual differences in the erythrocytes of the mule than in those of the horse. Likewise, there are differences between different donkey sera, but a given serum behaves in a similar manner to horse and mute corpuscles.

We find thus, that also in this instance mule blood resembles, on the whole, horse blood; but to a certain degree an influence of the donkey is noticeable and it modifies the inheritance in the mule. We may furthermore conclude that there are differences in individual mules. Of two mules, the one inherits a characteristic from the horse, while another inherits the corresponding characteristic from the donkey; yet both are equally mules. The hybrid character "mule" is therefore distinct from and independent of those individual characteristics which differ in different mules, some of which resemble, as far as this particular characteristic is concerned, more the horse, while others resemble more the donkey. The species differentials that distinguish horse, donkey and mule are definite, although some of the mosaic characteristics of individual horses and donkeys may vary, and the composite of these characteristics also differs in individual mules.

As to the analysis of mule blood by means of immune sera, Landsteiner and Van der Scheer found that while it might be possible to use the precipitin and complement fixation tests for the differentiation of the serum proteins of these three types of animals, the differences established by such methods

were found also in the cells of other tissues and organs. In the first named possibility we would have to deal with species-specific organ or tissue differentials. However, of special interest is the formation, in the hybrids, of a new agglutinogen, which is not present in the erythrocytes of either parent. This would indicate that new combinations of genes may give rise not only to corresponding combinations of substances which are present in the parents, but also to new substances which are not represented by any of the genes as such, but which depend upon the way in which the genes are sorted out.

In these various experiments we have to deal with different generic or species (or race) differentials in the parents and with combinations of such differentials in the hybrids. In the hybrids we note in all instances characteristics transmitted from both of the parents. In the mule, as well as in the hybrids between Ring dove and Pearlneck dove, it was observed that the hybrid red corpuscles contained combinations of the parental characters, either in smaller amounts or in the same complete assortment as the erythrocytes of the parent species. On the other hand, it may be recalled that by means of serological methods Todd found that the individuality differentials of children within the same race and species resembled either those of the father or those of the mother.

Furthermore, it would seem that some characteristics of the hybrids are constant and common to all individuals of the hybrid generation, while others are variable and may differ in different hybrids derived from the same parents. To the latter class belong, for instance, the blood-group characteristics; neither these nor their precursors can, as such, constitute or be a significant part of the species or hybrid differential. Also, the individual hybrids between domestic and wild Brazilian guinea pigs varied greatly in certain characteristics other than in those determining species or race, and behaved in this respect similar to the hybrids between inbred families of rats and guinea pigs when they were analyzed by means of transplantation; here also, all kinds of quantitatively graded, intermediate conditions could be established in the transmission of the individuality differentials.

An experiment similar to those reported in this chapter and dealing with animal species has more recently been performed by O. Moritz with plant species. He crossed Berberis empetrifolia with Berberis Darwinii and thus obtained the species hybrid, Berberis stenophylla. He then sensitized animals by injecting extracts from the leaves and young shoots of these three kinds of plants and by means of the anaphylaxis reaction he could show that the hybrid antigen contained constituents of both parent species. This experiment represents only a beginning in the analysis of plant hybrids by serological methods, but the results so far indicate that similar modes of distribution of the parent differentials in species hybrids will probably be found in plants and in animals.

cytes of the hybrid either did not possess all the antigens of the parental genera, or they did not possess them in the same quantity as the corpuscles of the parents. An additional interesting observation was the following: when the anti-hybrid rabbit serum was absorbed by the erythrocytes of both parents in succession there still remained in the immune serum a remnant of agglutinin, which could not be removed by such absorption; and furthermore, after absorption of the anti-hybrid immune serum by the red corpuscles of one of the parents, there still remained a greater amount of agglutinin against the erythrocytes of the hybrid than against those of the other parental genus. Irwin concludes that a new antigen (agglutinogen) must have developed in the erythrocytes of the hybrid as a result of fertilization, and that a new agglutinin may thus be produced in the immune serum. A combination of the gene sets belonging to the two parent genera would, therefore, give rise to something different from both component sets. Furthermore, it may be stated that the haploid number of chromosomes present in the germ cells of each of the parents is evidently able to produce in the hybrid almost the same amount of antigenic substance as the diploid number does in each of the parents.

Subsequently, Irwin and Cole investigated, by similar methods, the agglutinogens in the backcross generation from hybrid F_1 (Ring dove X Pearlneck) to Ring dove parent ("one-fourth Pearlneck"). In addition, the "one-eighth Pearlneck" backcross generation was studied; these were obtained by mating the one-fourth Pearlneck backcross a second time to a Ring dove parent. In these backcross hybrids a separation of the pearlneck genes took place, so that all possible random combinations were found, according to the rules of Mendelian segregation. The presence or absence of Pearlneck agglutinogens in the two backcross generations was tested as usual by the anti-hybrid F_1 rabbit serum which had been exhausted once or twice by various types of erythrocytes. The results showed that in the Pearlneck erythrocytes multiple, and at least ten, agglutinogens are present, which are distributed in a specific way in the backcross birds, so that each individual can be differentiated from the others, if a sufficient number of agglutination tests are made with anti-F_1 hybrid (Ring dove X Pearlneck) rabbit serum, after certain agglutinins have been absorbed with various kinds of erythrocytes. Besides, there was present in many, but not in all, of the backcross individuals the newly formed hybrid, agglutinogen, which did not exist in the erythrocytes of either parent, but formed as a result of the union of the genes of both parents in the F_1 generation.

However, these results were obtained only if immune serum from a certain rabbit was used for these tests. An immune serum from another rabbit might have given different results, and it is conceivable that if the immune sera had been used from a different species, additional differentiations would have appeared and the number of agglutinogens found in the Pearlneck erythrocytes would have been still further increased. Moreover, the question may be asked as to whether these species-specific multiple agglutinogens which were present in the erythrocytes were peculiar to these cells, or whether they

chicken serum, the blood of young chickens did not contain an active antigen which, when mixed with the precipitin of the immune serum, induced formation of a precipitate. Kritschewski noted that a substance obtained from nine-week-old tadpoles of Rana esculenta can be differentiated in its antigenic function from the substance of adult frogs by means of the complement fixation test, and he likewise observed that the Forssman antigen, which is present in the erythrocytes and organs of adult chickens, is not yet present in the egg and in very young embryos, but that it forms four days after the beginning of segmentation, when a more advanced embryonal stage has been reached.

These experiments, indicating that antigens develop only gradually during embryonal life, agree with the findings mentioned previously concerning the blood-group differentials, which seem to begin to form prior to the sixth month of pregnancy, but reach their full development only at about the time of puberty. It is necessary to select an especially active isoagglutinin in order to effect the agglutination of young as compared with older blood corpuscles. Likewise, the two different varieties of the A differential, A and A_1 (Thomsen), or A_1 and A_2 (Landsteiner), which differ in their binding power for isoagglutinin, gain their full strength only gradually with advancing childhood. While thus, in the course of time, the blood corpuscles acquire properties which enable them to bind a greater quantity of isoagglutinin, the difference in the ability of the A and A_1 corpuscles to combine with isoagglutinin remains preserved also at later periods.

Similarly, according to Thomsen, the full development of the isoagglutinin occurs only sometime between the fifth and tenth year of life, while in old age it may decrease again (Schiff and Mendlovitsch). Also, the natural amboceptor and complement of hemolysin, as well as the corresponding substances acting with bacterial substances, are not yet present in the earliest embryos, but form as embryonal life progresses in chicken and cattle (Sachs, Rywosch). In the chicken embryo, antibodies first appear after the twenty-first day. In very young swine embryos only very small amounts of complement, hemolysin-amboceptor and opsonin are found; these increase during embryonal development (Sherman). In this connection we may also cite the observation that while in the Freund-Kaminer test normal adult human sera are able to dissolve cancer cells, the sera of human fetuses do not yet possess this ability, and resemble in this respect the sera of persons afflicted with cancer.

It is well known that different species of animals differ very much in their power of resistance to certain bacteria and toxins. Similarly, the reactivity of young organisms to various toxins and bacteria may differ from that shown by adult organisms. Thus, according to Camus and Gley, the erythrocytes of newly-born rabbits are more resistant to eel serum than those of older individuals. Newly-born chicks and rabbits are not sensitive to arachnolysins and are less sensitive to cobra toxin. Heterohemolysins may be found in smaller quantity in younger than in older individuals, the amount of amboceptor as well as of complement being less in younger organisms. These observations agree with the findings in other experiments of Sachs, in which he

Chapter 5

On the Differences between the Reactions of Foetal or Newborn Organisms and of Adult Organisms Against Strange Differentials as Established by Serological Methods

In a preceding chapter we have discussed the differences in reactions between very young and older guinea pigs or of embryonal organisms against various homoiotransplanted tissues. The reactions against foetal or embryonal tissues have also been studied. In general, the reactions on the part of very young organisms were definitely diminished in intensity. As far as those of young guinea pigs towards homoiotransplants are concerned, this difference is due especially to a diminished intensity of the host connective tissue response towards the graft. The lymphocytic reaction may be quite pronounced, although it may appear at a relatively late date; this may be due to the fact that the transplant, being less injured by connective tissue, is able to exert a more marked effect on the lymphocytes. We have also referred to the transplantations of heterogenous tumors into the allantois of developing chick embryos, where at early stages the reactions against hetero-differentials are lacking. Similarly, it has been shown by various investigators that the reactions against transplanted tumors may not be so great in newborn animals and in early life as later. Blumenthal has shown that early stages of embryos do not yet contain fully developed organismal differentials.

It is of interest to compare with these findings the data supplied by serological methods. Roessle immunized rabbits with mammalian and avian embryonal tissues and found that these tissues were just as effective as antigens as were red corpuscles of adult animals in the production of hemolysins and also of agglutinins; there was no difference in this respect between embryonal and adult tissues. On the other hand, injection of pig embryo did not lead to the production of precipitins. The subsequent experiments of Braus also showed that injection of larval and embryonal amphibian tissues into rabbits did not lead to precipitin formation for either foetal or adult tissues, while injection of adult tissue produced precipitins which reacted with adult but not with embryonal tissue. He showed furthermore, that even tissue from an advanced stage of embryonal development which, when serving as host, no longer permitted a heterotransplant to grow, did not yet elicit the production of precipitin. We see, then, that while embryonal tissue may not possess antigen sufficient for precipitin formation, it may possess antigen which is able to call forth the production of hemolysins and agglutinins. Similarly, Uhlenhuth found that while serum of adult chickens gave a positive reaction with anti-

to any extent with the production of hemolysins following subcutaneous injections of sheep red blood corpuscles. In more recent investigations Jules Freund as well as Leona Baumgartner noted that production of agglutinin, hemolysin and precipitin, interacting with bacteria, erythrocytes or proteins, is less intense in very young rabbits, and according to Baumgartner, the avidity of immune sera for antigenic bacteria may be diminished. Furthermore, the skin in very young rabbits is less sensitive than in adult ones, and in young guinea pigs the skin likewise reacts less actively to tuberculin. However, as stated, at present we cannot entirely exclude the possibility that in some cases a previous infection with a homologous or heterologous microorganism may have caused an increased resistance against certain viruses or bacteria.

As to the mode in which a physiological maturation of cells leads to an increased resistance, Hirszfeld is inclined to attribute it to an increase in affinity of cells for certain toxins, taking place either during a normal biochemical maturation process or as the result of the previous activity or microorganisms and their toxins. Such an increase in affinity of cells for a certain antigen is also assumed to be the factor causing anaphylactic reactions. Thus during the process of immunization there may exist, side by side with the production of antibodies, an increased sensitiveness to the action of toxins. If children, negative to the Schick or Dick tests, who are allergic to the toxins of diphtheria bacilli or of streptococci, are actively immunized by the injection of these respective microorganisms or their toxins, their skin may for some time react positively to the local injection of the toxins and thus a change may take place, which is apparently due to an increase in the affinity of certain cells for the products of these bacteria.

From a purely chemical point of view, very little is known as to the changes occurring in cells with increasing age, although it has been shown that there are alterations in the water content and in the amount of calcium and cholesterin or its esters held by certain tissues during the process of ageing. Furthermore, Kossel has shown that during maturation of the sperm cells their constituent proteins undergo definite variations as far as the quantitative distribution of the amino-acids and diamino-bases (histidin, lysin, arginin) is concerned. According to Schenk, the character of the globin in the hemoglobin changes during the ageing of the erythrocytes or in persons affected by pernicious anemia. Thus it becomes conceivable that the mode of reaction of certain cells to homoiogenous sera or to toxins may differ under various conditions, and especially at different ages and during the process of immunization; but the chemical character of the factors underlying the change in the mode of reaction is not definitely known.

It may then be concluded that the differences in reaction towards homoiogenous or heterogenous tissues which organisms show at different stages of development and at different ages are not an isolated phenomenon, but are the expression of changes in reactivity to various types of foreign substances, especially to those of a toxic character. In general, both the reactivity of cells against foreign substances and the ability of tissues to produce immune substances against them is lacking or diminished in young organisms. Presumably

showed that immune hemolysins produced against erythrocytes of adult chickens, beef, rabbits and guinea pigs are without injurious effect if they are injected into the circulation of embryos or of newly-born animals of these species. Similarly, very young children often prove negative to the Schick and Dick tests, their blood being free from antitoxin. On the contrary, we may find in young organisms a lack of resistance to certain bacteria and viruses, to which adult organisms may be definitely resistant. Thus there seems to be an increase in the resistance to poliomyelitis with advancing age, as manifested by the fall in morbidity and the rising level in the therapeutic efficiency of blood serum. In the serum of adult Rhesus monkeys a substance neutralizing the poliomyelitis virus may be observed, while it is lacking in immature monkeys. Such increased resistance has been attributed by some investigators to a preceding latent or very mild infection with this disease. But Jungeblut and Engle could show that such a change may occur even in monkeys which have been kept isolated and that it is therefore probably not due to a previous infection.

Likewise, young rats are much more susceptible than are adult rats to inoculation with pneumococci. The blood of almost all humans is destructive for pneumococci of type II, while that of very few persons possesses the power to destroy type I. The blood of an intermediate number of individuals destroys type III. In the blood of young children, one to fifteen months old, this ability of a mixture of serum and leucocytes to kill pneumococci is rarely observed (Robertson and Sia); with advancing age its frequency increases, but in the aged it is again rarely present. As in the case of poliomyelitis, some investigators attribute also this increase in resistance with advancing age to a preceding latent or mild infection with the specific organism, but recently the suggestion has been made that an infection with a different and perhaps non-virulent organism which has certain antigens in common with the pathogenic one, may be responsible for such an effect. But this interpretation is not sufficient to explain all observations, although it may apply in some instances. Other investigators, Friedberger, Hirszfeld, Jungeblut and Engle, assume therefore that a gradual physiological ripening, due to a biochemical change in cells or tissues, is the cause of this altered mode of reaction in older age. On the whole, this explanation appears more probable and it seems to hold good, for instance, in the case of the blood-group differentials and in other instances already mentioned. A maturation immunity develops in mice, with advancing age, to the virus of vesicular stomatitis. Mice two weeks of age, succumb to intramuscular injection of this virus in almost 100 per cent. With increasing age, the resistance gradually increases. Mice older than six weeks are completely resistant to intramuscular injection, but not to intracerebral administration of this virus (Olitsky, Sabin and Cox). A barrier to the further progression of the virus seems to develop at the myoneural junction. In favor of the theory of a maturation resistance of tissues, it may also be stated that an active immunity is very difficult to produce against certain cells and toxins in very young animals. This fact has been demonstrated by the experiments of Famulener, who found that young kids did not respond

stages, but merely that the onto[illegible]
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cert[illegible] [illegible]. Furthermore, the organs and
tissu[illegible] [illegible] of phylogenetically higher, more differentiated organisms may resemble the organs and tissues of embryonal, and, perhaps, even of adult, phylogenetically more primitive forms. But, the embryonal organs and tissues of the higher organisms differ from those of phylogenetically less developed species, in that the former possess specific precursor substances of the organismal differentials present in the corresponding adult forms, which are lacking in the phylogenetically lower embryonal or adult organisms.

the production of immune substances presupposes the power of the tissues to respond with primary reactions against the foreign material. The chemical differentiation is not yet completed in fetal or newly-born organisms, as is evidenced by their diminished power to act as antigens. Perhaps this lack of available antigen in certain cells may be responsible for a diminution in their binding power for toxic substances and in their ability to react against the latter. Moreover, it seems that, as we have seen in the case of the blood-group agglutinogens, differentials may develop in certain cells before the mechanisms have developed which lead to the production in the serum of specific substances interacting with those differentials. Such observations permit the conclusion that in the embryo and fetus some substances, which are present in the adult organism and which may function as antigen, are lacking, and likewise, that the earlier ontogenetic states have not yet acquired the full power to react against and to neutralize strange and toxic substances. In agreement with this interpretation are certain experiments of Theobald Smith and R. B. Little, who noted that newborn calves are prone to acquire a generalized infection with colon bacilli; this can be prevented if the calves are fed colostrum or receive the serum of a lactating cow. The deficiency in globulin in the blood of newborn calves prevents the production of agglutinins in these animals (Orcutt and Howe). P. Cannon suggests that the availability of globulin for the production of immune substances is the essential factor on which depends the ability of an animal to respond with immune processes to injurious interferences. Globulin is relatively deficient in very young, and again in old individuals and also under unfavorable conditions of nutrition. While this factor may play an important role in determining the degree of resistance of an animal to an injurious condition, it is probably not the only one which is active.

But there exists, on the other hand, also some evidence which indicates that the embryo and fetus, and even the organism at the time of birth, may possess substances which are able to act as antigens and which are not possessed by the adult organism. Thus Lockemann and Thies, and Graefenberg and Thies, found that it is possible to sensitize adult rabbits with the serum of rabbit fetuses, and that a second injection of such serum causes anaphylactic shock in the sensitized animals; even the mother can thus be sensitized against the blood serum of its own fetuses. It appears, furthermore, that during the later stages of pregnancy, rabbits as well as guinea pigs become naturally sensitized against a substance in the blood of their fetuses, the pregnant rabbit and guinea pig being sensitive to the injection of the blood of newborn animals belonging to the same species. However, in addition to these effects, toxic substances of another kind may apparently be active in pregnant animals; it has been stated that the latter are sensitive also to the injection of the blood serum of pregnant animals belonging to the same or different species, while normal guinea pigs seem to be more sensitive to the serum of puerperal than to that of pregnant animals.

We do not need to conclude, as have some investigators, that the earlier ontogenetic stages represent different and phylogenetically more primitive

some way connected with each other, differences in organismal differentials, which distinguish individuals or species, being associated in a graded way with differences in organ differentials and, in general, with differences in the mosaic structure of the organism of which these organ differentials form a part. In the embryo, the organismal differentials, as well as the organ differentials, are not yet fully specialized; in both cases only the more fundamental differentials, the precursor substances of the finer differentials, exist. Thus the organ differentials at a certain embryonal stage may be represented by the differentials of the germ layers. Furthermore, the organizer (inductor or evocator) substances in their origin are intimately connected with the mosaic characters of the individual; they are produced in differentiating organs or tissues and initiate further differentiation in other organs or tissues. The organ differentials represent, therefore, the specific structures and functions of the tissues and organs within an organism; they are intraorganismal differentials.

As we have stated previously, in this strict sense the organ differentials represent the specific factors which are common to the same types of organs in individuals belonging to the same species and in species belonging to the same order or class; but in making these distinctions between homologous organs and tissues, it must be understood that finer differences exist between tissues in the same organism than is usually assumed. When we differentiate between certain organs and tissues in an organism we use for this purpose, as a rule, very obvious anatomical and histological peculiarities. Thus we distinguish between organs, such as liver and kidney, and between tissues, such as epithelium and connective tissue; among the epithelial tissues we distinguish still further between stratified and glandular epithelium, and among the connective tissues, between the ordinary collagenous and the cartilage and bone-forming connective tissues. However, further analysis shows that these are rather crude distinctions, that in reality much finer ones exist between different tissues, which, on the basis of morphological criteria, we are accustumed to consider as possessing essentially the same character. As we have mentioned on previous occasions, biochemically the connective tissue of the uterine mucosa differs from that of the Fallopian tube and vagina, and even within the different parts of the cervix there are graded differences in the connective tissue, as is shown by the response of these tissues to the lutein and follicular hormones. Fibroblasts differ biochemically in different areas of the embryo as to the amounts of acid they produce and also in their resistance to acid and in their proliferative power. Furthermore, Huggins has shown that the regenerating epithelium of the bladder has the power to transform the fibroblasts of certain organs, but not those of others, into osteoblasts, which are then able to produce bone. Apparently the epithelium of the bladder gives off a contact substance, which acts as an organizer and transforms only connective tissue cells of some organs into osteoblasts. As these examples show, the differentiation of various tissues is a much finer one than is assumed on the basis of ordinary morphological criteria; but such finer differentiations have not yet been subjected to serological tests and these would presumably be unsuccessful on account of

Chapter 6

Organ (Tissue) Differentials and Their Analysis by Serological Methods

WE HAVE DISCUSSED the difference existing between the organismal differentials and the mosaic characters which compose the individual. It is the latter with which Mendelian heredity and also embryology have so far been almost exclusively concerned. The organismal differentials are common to all parts of an individual, except perhaps certain paraplastic structures; they thus differ from the mosaic characters which distinguish the various organs and tissues in the same individual, and which are about the same in organs and tissues of the same type in two different individuals with distinct individuality differentials. As we have seen, it is possible to analyze the organismal differentials not only by means of transplantation but also by means of serological experiments, one of these two methods being preferable in certain ranges of the spectrum of relationship, the other in other ranges. Similarly, it is possible to analyze by serological methods the organ differentials, those chemical factors which are the same in analogous organs in different individuals, but which differ in the different organs within the same individual. Moreover, it has been found that there are a few organs or tissues in which, under certain conditions of experimentation, species differentials cannot be demonstrated by serological methods, but in which organ differentials can be distinguished by these means. The first example of this kind observed and the one best known is the lens of the eye. Uhlenhuth found that this organ can function as antigen and lead to the production of anti-sera, which, however, react about equally against the lens substance of mammals, birds, reptiles and amphibia, while they do not affect other organs of the species whose lens was used for immunization.

Organ differentials are, therefore, factors inherent in organs or tissues; they are very similar in the corresponding organs and tissues of different individuals and species and represent the structural and functional characteristics of these organs and tissues, while the organismal differentials are the substances which distinguish organisms as such from one another, and are the same in different organs and tissues of the same individual. The organ differentials represent what is different and distinct in different parts of the same organism, while the organismal differentials represent what is common to different parts of the same organism but differs in analogous organs and tissues of different individuals and species.

Potentially, both organismal and organ differentials are present in the fertilized egg in the form of precursor substances. These differentials are in

precipitation method, especially in combination with specific absorption, has been used most commonly in the analysis of such antigens.

(2) If the immune serum directed against a certain organ, or against a characteristic substance derived from this organ, reacts with the analogous organ or substance not only from the species which served as the donor of the antigen, but also from other species more nearly related to or even farther distant from the donor species, then we assume that these immune sera and the corresponding antigens are organ-specific. Thus, as stated, the immune serum directed against the lens of mammals may react about equally well with lens material from birds, reptiles and amphibia, and even, although not quite so well, with lens substance of fishes; in this case we have therefore predominantly to deal with organ differentials.

(3) If the immune serum, directed against a certain organ, should react also with blood serum or some of its constituents, or with other organs of the donor species, the latter reaction must at least be weaker than the one which takes place with the organ which served as antigen for the immune serum. In some cases the reactions are graded in accordance with the graded relationships obtaining between the antigen-furnishing organ or tissue and the other organs or tissues of the donor species. Thus immune serum against guinea pig erythrocytes may react not only with erythrocytes, but also with leucocytes and spleen tissue of the guinea pig, but not with other organs of the guinea pig, nor with the corresponding rat cells; and the immune serum against brain may react also with testis, but not with other organs. If an immune serum reacts not only with constituents of an organ which served as antigen but also with a certain constituent of the blood, it does not necessarily mean, therefore, that an organismal differential served as antigen—although it may have this meaning—but it may mean in some instances that the splitting of very complex material, characteristic of an organ, into somewhat more elementary substances may lead to a relative organ-specificity, which allows for the presence of similar substances in certain other organs or tissues, or in the blood of the same species. The chemical constituent common to two or more organs and, perhaps, to blood, which is responsible for the joint reaction of these organs or of the blood with the immune serum primarily directed against one particular organ, would not, in this case, be a part of the organismal (species) differential, but of the differential of the organ used for immunization, as well as of the differentials of certain other organs or tissues.

It is possible in some cases to increase the specificity of the immune serum against an organ differential and to diminish or destroy entirely its reaction with organismal differentials by boiling the antigen before injecting it. In this way the organismal differentials, which concomitantly with the organ differentials might serve as antigens, are injured in their antigenic power to a much higher degree than are the organ differentials. Furthermore, if the test reaction is carried out with an alcohol extract of the organ which served as antigen, instead of with native or boiled antigen, the organ-specificity is

the present lack of satisfactory serological methods for the detection of these organ or tissue differentials.

The organ differentials develop in the embryo and undergo a predetermined sequence of transformations. As a rule, the organismal differentials, or their precursors, are present in the developing organs and tissues; however, certain endproducts of these transformations of organ-forming substances may lose a part, or even all, of their organismal specificity, as occurs for instance in the case of some enzymes and many hormones and related substances. Also, the endproducts of tissue differentiation, such as keratin and lens fibers, may lose entirely or in part the finer organismal differentials, while the organ differentials retain their full strength; this applies only when certain serological tests are used as indicators for the organismal differentials. Because of the relative predominance of the organ differentials and the diminution in the significance of the organismal differentials in certain organs or tissues, it is possible to demonstrate by serological tests, organ specificity against tissues and substances derived from the same species when it is difficult to demonstrate individuality and species differentials. Homoiogenous lens, spermatozoa, keratin, thyreoglobulin, fibrinogen, and even insulin, may function thus as organ, tissue or substance antigens. Likewise casein from goats' milk and the albumin of chicken egg may serve as such antigens in goats and chickens, respectively. In a similar way, Schwentker and Rivers produced antibodies against rabbit brains in rabbits, not only by the use of a combination of rabbit brain extract and pig serum, but also autolyzed or otherwise pathologically altered brain as such could serve as antigen. Substances of a homoiogenous nature may function in serological tests as antigens if they are abnormal or if they do not occur in the circulation under ordinary circumstances.

Both organismal and organ differentials develop thus by a chemical epigenesis in the course of phylogenetic and ontogenetic development. However, while the organ differentials or their precursors not only undergo very far-going, specific changes during embryonal development, but are also readily accessible to experimental modifications, the organismal differentials or their precursors seem to be fixed; so far it has not been possible by experimental means to transform one organismal differential into another, at least in higher organisms, while it has been possible to change, experimentally, the mode of development and the transformations of organs and tissues.

We are here concerned only with the serological methods employed for the analysis of organ differentials. As to the criteria that can be used for this purpose, we assume the presence of organ differentials, in contrast to organismal differentials, under the following conditions:

(1) If an immune serum, e.g., one against fowl egg, differs in its reaction qualitatively or quantitatively from one against blood serum of fowl, or against other organs, tissues or substances derived from the same individual or species, we conclude that an organ differential was involved in the antigenic action which gave rise to the formation of the immune serum. The

specificity of the lens of the eye; from the lens substance, partial antigens, two crystallins, may be separated, and also against these specific precipitins can be produced, which are the same irrespective of the species from which the antigens are derived (Hektoen and Schulhof); this is an observation which is in accordance with what has been found in the case of the precipitins against the lens as a whole. However, these two crystallins are related to each other, because cross-immune reactions between them do occur. We see, then, that in this instance the organ specificity can be reduced to the specificity of certain substances derived from these organs. But in a preceding chapter we have seen that according to Defalco it is possible, by means of the precipitin reaction, to demonstrate in the lens of birds the presence of species differentials.

The brain behaves in a similar manner to the lens; it also shows a very pronounced organ specificity, which may or may not be associated with species specificity. However, as stated, immune serum against brain reacts equally well with testis (J. H. Lewis). Also, vitellin obtained from egg yolk, as well as casein and thyreoglobulin (Hektoen) are organ- or rather substance-specific material. Anti-thyreoglobulin sera do not react with globulins from other organs.

In other cases a more graded specificity exists. Thus immune serum against egg albumin reacts also with albumin from fowl serum; yet both these albumins can be distinguished by means of quantitative tests. Fibrinogen and the globulins of chicken plasma are immunologically nearly related to each other; but they can be distinguished by means of quantitative tests with immune sera; they are very different from the albumins of fowl's egg or fowl serum. Similar relationships are found between serum globulins of mammalian organisms (Hektoen and Welker). As Dale and Hartley, as well as Doerr and Berger, have shown, the serum proteins exhibit two kinds of specificities: (a) The species-specificity, which depends upon the character of the organismal differentials; this is the same in the various plasma proteins from the same blood. (b) The fraction-specificity, so-called by Doerr, which corresponds to organ-specificity. Each mammalian serum protein can be distinguished from another serum protein derived from the same individual or species by means of the anaphylactic reaction. Likewise, Bence-Jones protein, which occurs in the urine under certain pathological conditions (multiple myeloma), is serologically quite different from the normal serum or plasma proteins. Immune sera against hemoglobin react with hemoglobin but not with serum proteins. Furthermore, the antihemoglobin sera are quite distinct in their reactions from immune sera against the stroma of erythrocytes; the latter contain hemolysin and hemagglutinin, in contrast to the antihemoglobin sera, which do not contain these two antibodies. In general, the organ proteins are distinct from the proteins of the bodyfluids, although the latter may be derived from certain organs. On the other hand, we have mentioned the close relation which exists between albumin from egg and from serum.

In lens and brain, lipoid substances seem to be at least partly responsible

intensified in certain instances. Serologically, organ differentials have been tested in vitro mainly by means of the precipitin or complement fixation reactions; organ differentials have also been tested in the living animal by means of specific cytotoxic effects following injection of immune serum against a certain organ, or by means of anaphylactic reactions.

In the large majority of cases where an organ differential has been shown to exist, the simultaneous presence of the organismal differential in the material serving as antigen could likewise be established, or at least made probable. A combination of these two antigens is indicated under the following conditions:

a. If an immune serum reacts not only with the organ or the substance used as antigen, but also with other organs or fluids of the same species, although to a lesser degree, this might be due to the presence of an organismal differential in the antigen; but, as stated above, this is not necessarily the case.

b. The conclusion that also an organismal differential is involved in the reaction is strengthened if the immune serum reacts with the analogous organs of other species in such a way that the reaction is the more intense, the nearer the species providing the antigen and the second species to be tested are related to each other.

c. If the immune serum reacts only with the organ of the donor species which served as antigen, but not with other organs or with the blood of the donor species, and if it does not react with the analogous organs of other species, then such an immune serum may or may not be directed also against the organismal differential. We may possibly have to deal with an immune serum specific for a certain substance which does not possess an organismal differential. But if the immune serum, while reacting most intensely with the antigenic organ of the donor species, reacts likewise, although more weakly, with other organs of the same species, but does not react with the corresponding organs of other species, then the material which served as antigen contains in all probability both organ and organismal differentials. The lack of a reaction with any other species in such a case may represent the end-stage in a series of reactions, in which the intensity of the reaction decreases more and more with the increasing distance in relationship between the antigen-providing species and the other species which are to be compared with the former.

Various organs, and substances derived from these organs, differ very much as to the degree of their organ- and substance-specificity. According to Fleisher, who used in vitro tests, the simultaneous presence of species-specific substances and of substances of a non-specific character in various organs complicates the demonstration of the organ- and tissue-specific substances which they contain. But quite apart from these complications, different organs actually seem to vary considerably in the readiness with which these organ-specific substances can be demonstrated. Thus, Fleisher states that it is very difficult to demonstrate them in the spleen, but that this can be more easily done in liver and kidney. We have referred to the marked

either in the extracts or suspensions of certain organs or in chemically defined substances obtained from and characteristic of such organs. We shall consider only certain of those substances concerning which there are on hand data sufficient for this analysis.

1. It is known that a comparison of the blood sera of various groups of animals, when tested by means of the precipitin reaction, indicates the degree of relationship of these animals within a somewhat limited range of specificity. We have furthermore seen that different serum proteins show a definite substance-specificity when tested with precipitin containing immune sera; but a specific reaction takes place in the latter case only if the antigen and the corresponding substance with which it is to be compared belong to the same or to nearly related species (Hektoen and Welker). Thus, immune serum directed against the serum globulin prepared from human serum reacts only with the globulin prepared from human or from monkey blood. Immune serum against fibrinogen prepared from mammalian blood reacts strongly with mammalian but only weakly with chicken fibrinogen, while conversely, anti-chicken fibrinogen serum reacts strongly with chicken and but little with mammalian fibrinogen. By means of absorption of the immune serum by the principal antigen, all the antibodies can be removed from the immune serum, but absorption with the corresponding antigens from other species removes only the special antibodies which are adjusted to the latter kinds of antigen, while the principal antibody, namely that which is directed against the fibrinogen of the species which was used for immunization, remains in the serum. Much finer are the differences between the albumins from the egg of different species. Here the investigations of Dale and Hartley, who used the anaphylactic method, at first seemed to indicate an identity between the crystallized albumins of fowl and duck eggs, but by the use of more refined methods Dale succeeded in distinguishing between these two substances also immunologically. Such a result suggests that in other cases as well, when apparently no immunological differences exist between two corresponding proteins from two species, such differences after all may exist.

These observations show that we may have to deal in these instances not only with organ differentials, but also with organismal differentials. Likewise with casein, it seems possible that a combination between a substance-(organ) and an organismal-specificity exists, although by means of immune reactions apparently no differences between the antigenic effects of different mammalian caseins can be established.

2. Hemoglobin possesses a distinct substance-specificity, corresponding to a tissue- or organ-specificity; the immune serum against this substance contains specific precipitins. However, an immune serum against hemoglobin of a given species reacts not only with the hemoglobin from this species, but also with the hemoglobin from nearly related species, but not with that derived from a distant species. Thus immune serum against cattle hemoglobin may react also with a solution of sheep hemoglobin, but not with a solution of hemoglobin from farther distant species; exceptionally, this immune serum reacts with human hemoglobin, but only if it is used in

for the organ-specificity displayed by these organs. According to Witebsky, organ-specific lipoids are present also in kidney and liver. In seeds of plants there occur alcohol soluble prolamins which are identical in very nearly related species and somewhat different in more distant groups (Wells and Osborne, Gortner). These may be considered organ-specific substances. We have referred to these investigations in a preceding chapter, but may discuss here certain points which relate to the problem under consideration. Wells and Osborne found that, as evidenced by the anaphylactic reaction, hordenin from barley and gliadin from wheat are similar to each other; likewise, gliadin and glutenin from wheat behave much alike immunologically. However, a guinea pig sensitized with gliadin reacts somewhat more strongly with gliadin than with hordenin. On the other hand, hordenin and glutenin are quite different, as far as their immunological reactions are concerned; it may provisionally be concluded that glutenin and gliadin are distinct substances and that the common reaction shown by them is due to a common radicle which they possess. But hordenin does not seem to have this common group, which may correspond to an organismal differential, though it possesses some radicle in common with gliadin, which is not shared by glutenin. This is an interpretation of the facts which would seem more probable than the assumption that we have to deal, in gliadin, hordenin and glutenin, with mixtures of different proteins.

Also, in the case of animals evidence has been found that the analogous proteins in different organs may contain different radicles which determine the organ-specificity and are associated with certain other characteristics of the protein which determine the organismal differentials. However, as to the character of these gradations in structure, interpretations may differ; it might, for instance, be assumed that the character of the radicle is approximately the same in nearly related species, but differs more strongly in more distant species, although some similarity may still exist in the structure of this radicle even in remote species. It is also conceivable that finer chemical groups are the same, or only very slightly different, in all nearly related species, but that the common basic radicle on which they have been superimposed is the same in nearly related species, but differs in more remote classes of animals.

The investigations we have mentioned may serve as examples of organ-specificity, the latter being due to substances contained in these organs and tissues; these substances are evidently the bearers of the orgao specificities. It is of interest that these organ-specific substances apparently represent either reserve foodstuffs or secondary cell constituents not exhibiting the most characteristic features of living matter, but constituting end-products of cell differentiation and specialization. Other substances of this kind are pathological in origin. How far the more labile constituents of living cells, as for instance certain nucleo-proteins, possess organ-specificity is as yet unknown.

We shall now take up somewhat more in detail the question as to what extent organ differentials may be associated with organismal differentials,

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ever, the characteristic crystal forms of the hemoglobins, which were studied by Reichert and Brown, correspond to primary differentials in transplantation and not to antigens which call forth immune reactions, with which we are here principally concerned.

There are other characteristics of hemoglobin which may serve to differentiate the species of animals and even individuals, but no definite correspondence has been shown to exist between these characteristics and the relationships of the species or individuals tested. In this regard, according to Anson, Bancroft and Mirsky, there are differences in the maximum spectrographic intensity of the bands of oxyhemoglobin in different species, but the distribution of these bands does not correspond to the relationships of the species. Measurements of the distances between the maximum intensities of the A bands of oxygen and carboxyhemoglobin showed individual as well as species differences, but the individual differences could be greater than those between species. In these respects these characteristics behaved therefore to some extent like the blood-group differentials, which are quite unlike the individuality differentials in their distribution.

3. Thyreoglobulin immune serum reacts specifically with thyreoglobulin, but not with globulins from pancreas and adrenal glands, and the immune sera against the latter substances react specifically only with the globulins from their respective organs. But it seems that in the case of thyreoglobulin the organ-specificity is not an absolute one and that this substance may react also with certain other proteins from the same species. There is an organismal differential present in thyreoglobulin, in addition to its organ- or substance-specificity. The immune serum against thyreoglobulin reacts in a graded way also with the thyreoglobulin from other species and in this case the range of associated secondary reactions is wider than in the case of serum proteins and of hemoglobins. Thus immune serum against thyreoglobulin of one mammalian species reacts in the greatest dilution with thyreoglobulin from the same species, but in stronger concentrations it reacts also with many other mammalian thyreoglobulins; however, the relative specificity, as manifested by the graded character of the reaction, does not seem to be present in every instance, probably owing to an organ- or substance-specificity, which the thyreoglobulins from many species have in common and which may sometimes cover up the organismal-specificity. Still, a species-specificity does exist, as is shown by the fact that immune serum against chicken thyreoglobulin reacts with chicken thyreoglobulin, but not with mammalian thyreoglobulin. As with hemoglobins and serum proteins, so too with thyreoglobulin, absorption of the immune serum by the thyreoglobulin which served as antigen removes also the antibodies against the associated secondary thyreoglobulins, while absorption with an associated thyreoglobulin removes only this secondary thyreoglobulin, but leaves the antibody against the principal thyreoglobulin intact. If for immunization heated thyreoglobulins are used as antigens, the resulting sera contain only organ-specific antibodies, but not antibodies against the organismal differentials (Witebsky). Similarly,

stronger concentrations. Likewise sheep and goat hemoglobins react with the immune sera against hemoglobin from either of these two species and immune serum for human hemoglobin reacts also with monkey (*Macacus rhesus*) hemoglobin. Immune serum directed against chicken hemoglobin may react with hemoglobin from turkey, duck and pigeon, although not with hemoglobin from goose. If immune serum against cattle hemoglobin is exhausted with cattle erythrocytes, all the antibodies against any kind of hemoglobin are removed, but if such cattle immune serum is exhausted with sheep or human corpuscles, only the antibodies against sheep and human hemoglobin, respectively, are removed. In this instance we have to deal with a phenomenon similar to that observed in the case of fibrinogen.

Heidelberger and Landsteiner demonstrated the specificity also of crystalline hemoglobin and were able to show that the precipitate which forms, when hemoglobin and its immune serum are mixed, is due to hemoglobin as such acting as an antigen and not to an adhering impurity. They furthermore found the specificity of hemoglobins derived from different species to be very great; thus horse hemoglobin immune serum reacts much more strongly with horse hemoglobin than with that of the donkey. In addition, the reaction was found to be substance-specific, the immune serum against horse hemoglobin reacting not at all or only very weakly with serum albumin from horse. However, according to Higashi, immune serum against chicken hemoglobin gives a reaction of equal or nearly equal intensity with hemoglobin of pigeon or sparrow; this, then, would indicate a restriction of the species-specificity of hemoglobin. Hemoglobin has, therefore, a marked organ-specificity and a definite although somewhat less marked organismal-specificity, comparable to that of serum proteins.

According to Ottensooser and Strauss, globin, which can be split off from hemoglobin, also has a similar organ- and species-specificity. Immune sera against horse globin and against horse serum do not give cross-reactions if the complement fixation is used as a test, but if precipitation is the test method, immune serum against globin from horse reacts also with horse serum as a whole, but not with albumin from horse serum, while immune serum against horse serum does not give a precipitate with globin. By preparing amino- and nitroglobin further structural specificities are produced. Anti-globin sera reacted with anti-nitro- and anti-aminoglobin sera, but the reciprocal reactions did not take place; anti-amino- and anti-nitroglobin sera reacted only with their respective antigens, but not with globin.

In these experiments the relationships of hemoglobins of various species were tested by means of immune sera and a substance- (tissue) specificity, as well as an organismal-specificity, was found; the antigens, within a certain range of accuracy, behaved in accordance with the relationships of the various species. Reichert and Brown had previously observed that also the structure of the hemoglobin crystals corresponded to the phylogenetic relationships of the species from which they were derived. These criteria did not, however, suffice for the differentiation of horse, donkey and mule hemoglobin. How-

specificity. This interpretation agrees with the findings of v. Szily, to which we shall soon refer.

However, that organismal differentials are still present in the lens is also indicated by the fact that injection of homoiogenous lens material in the rabbit does not, as a rule, lead to the production of antisera, but it is necessary for this purpose to use heterogenous lens substance, and conversely, in a rabbit sensitized with strange lens material its own lens cannot call forth an anaphylactic reaction. This observation is in agreement with the demonstration of individuality differentials in lens tissue by means of cellular reactions. Still, according to Guyer, it seems that a guinea pig can be sensitized to strange lens material by injuring the animal's own lens. It is possible that in the case of the lens a very pronounced organ specificity covers up the more finely graded organismal differentials and allows only the very coarse ones to become manifest. In agreement with this interpretation would be the experiments of Defalco, who obtained species-specific precipitins for the avian lens.

6. Two different types of organ-specific constituents have been demonstrated in the brain by Witebsky and his collaborators. Witebsky and Steinfeld showed that there are (1) alcohol soluble, coctostable substances. The antigen, or rather hapten, present in an alcohol extract from the brain of a given mammalian species, reacts not only with the immune serum produced against the brain extract from this particular species, but also with those of all other mammalian species. It is therefore to a very high degree organ-specific, and to a much lower degree, or not at all, species-specific. Similar organ-specific, alcohol soluble differentials can be shown to exist in the posterior lobe of the hypophysis and in the medulla of the adrenal gland. (2) In addition, there are demonstrable in brain suspensions, heat-sensitive substances, which are not soluble in alcohol and which react only with the immune serum against the brain of the species from which this organ was derived; hence, beef brain suspensions react only with immune serum against non-heated beef brain suspensions. These differentials, which are presumably of a protein nature, are therefore not only organ-specific, but also, to a high degree, species-specific. The immune serum prepared against non-heated brain suspensions reacted in some instances also with alcohol soluble antigens; but such a reaction did not take place if the corresponding immune serum against certain other organs, such as the epiphysis, were tested in a similar manner.

While in some cases, according to Witebsky and Lehmann-Facius, boiled or alcohol soluble organ extracts seem to be better suited than watery, non-heated extracts for the demonstration of organ-specific constituents, this apparently is not true in all cases. Thus it is possible to distinguish by means of complement fixation between brain and epiphysis, if we use immune sera prepared against the unheated, water soluble antigens; but immune sera against the heat stable, alcohol soluble substances in epiphysis and brain do not make possible the distinction between these two organs. The alcohol soluble substances in brain and epiphysis are evidently identical, or at least

Witebsky found that the globulin of adrenal glands and pancreas lose their organismal differentials as the result of the heating but retain their organ differentials.

4. If we use whole cells or pieces of organs for immunization, we find again a combination of organ and organismal differentials present in the material serving as antigens. If rat leucocytes are injected into an animal belonging to a different species, the immune serum reacts most strongly with rat leucocytes and more weakly with kidney and liver of rat. This indicates a relative organ-specificity; but in addition there is noticeable also a reaction with guinea pig and rabbit leucocytes, which is weaker, however, than the reaction against rat leucocytes. This graded character in the reactions indicates the presence of organismal differentials. Forssman differentials are also found in leucocytes (Witebsky).

5. In the lens there seem to be only organ-specific differentials, yet a closer analysis indicates the presence also of organismal differentials. This was shown by the transplantation experiments of Fleisher, and the recent experiments of Blumenthal indicate even the presence of individuality differentials. Serological tests also suggest the presence of organismal differentials, at least of the very coarse ones. Thus immune serum against mammalian lens manifests a weaker reaction against fish lens, or the reaction against the latter may even be lacking altogether. Conversely, the anti-fish lens serum reacts more strongly with fish lens than with mammalian lens (Hektoen and Schulhof). By means of absorption tests it can be shown that each type of lens, fish as well as mammalian, binds its own immune serum quota in a specific manner and leaves the other fraction behind in the serum.

According to Krusius, a guinea pig sensitized against a mammalian lens reacts only very weakly against fish lens, and not at all against the lens of the eye of cephalopods. Likewise, the observation of Krusius, that if animals are sensitized with the complete lens of a certain species, there may take place a slight reaction also with the serum from this species, indicates the presence of organismal differentials. As Krusius points out, this reaction is probably due to the effect of the outer layer of the lens, which shows as yet a less fargoing tissue differentiation than the inner lens fiber material. With increasing tissue differentiation of this, as well as of other organs, the organismal differentials seem to become less marked or to disappear in the end, while the organ differentials become more pronounced, at least as far as the serological tests indicate. It is the transformation of the capsular epithelium into lens fibers which brings about this change. A similar change takes place, according to Krusius, when the epidermis undergoes keratinization. The species-specificity depends, therefore, apparently upon the presence of primary tissue proteins, while the organ-specificity depends upon a modification of the primary tissue proteins comparable to the introduction of a NO_2 group into the protein molecule in the experiments of Obermayer and Pick. The keratin of horse hoof and of human hair shows accordingly, in anaphylaxis experiments, an organ-specificity in addition to a species-

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which was tested with the immune serum, was derived from cancer of the same organ as that which served as antigen for the preparation of the immune serum, than if cancer of a different organ was used; but positive results were obtained in many cases also with autolysates from cancer originating in a different organ. Likewise, the serum of cancerous patients reacted with such immune sera, and in this case also there was an indication of an organ specificity. No reactions were found, as a rule, with the sera of non-cancerous persons. It seems, then, that in carcinoma a protein antigen is present, which possesses a certain degree of organ specificity combined with species specificity.

9. It is possible to produce specific immune sera also against spermatozoa, or, rather, against spermatic fluid, as well as against testicle and epididymis. According to Ohki, the immune sera against the latter organs owe their origin to spermatozoa, or to precursors of spermatozoa, which are found in the tubules of the testicle and epididymis. He finds that the anti-spermatozoa sera produced in rabbits react most strongly with spermatozoa from the same species which served as donor, but that a weaker reaction may take place also with the sperm-antigens from heterogenous species. Even between spermatozoa of birds and mammals and their immune sera an interaction may occur. Both precipitin and complement fixation reactions were used in these tests. They are, on the whole, specific for the spermatozoa present in testicle and epididymis, although the immune sera against these cells may also react with the blood serum of the donor species; but the latter kind of antibodies can be removed by means of selective absorption, following which the antisperm antibodies alone remain in the immune serum. It is also possible to remove by means of specific absorption, through previous addition of spermatic fluid, the antisperm fraction of the immune serum; but while, in this case, according to Hektoen and Schulhof, the antibodies against blood serum are also removed, according to Strube the precipitins for blood serum remain intact in the immune serum. It may then be stated that distinct antibodies may be produced against a spermatic fluid and against a serum fraction present in the antigen. On the other hand, according to Ohki, it is possible to obtain immune sera against spermatozoa which do not react with the serum of the donor. If we accept the conclusion that it is really the spermatozoa, as such, against which the immune sera are produced, we should have to assume that the spermatozoa contain both organ and organismal differentials, or rather their precursor substances. They would contain organismal differentials or their precursors, because the immune sera react most strongly with spermatozoa of the donor species; they are organ-specific because an immune serum against mammalian spermatozoa reacts also with avian spermatic fluid. While also in this instance the organ differentials seem to be more prominent than the organismal differentials, they are less so than the differentials of the lens of the eye. It is, furthermore, of interest that according to Ohki not only heterogenous spermatozoa may serve as antigens, but also those of homoiogenous, or even of autogenous, origin.

so similar that they cannot give origin to organ-specific immune substances. On the other hand, certain water soluble, heat sensitive substances, presumably of protein nature, in brain and epiphysis represent organ differentials which do permit the distinction between these two organs. However, while these two kinds of immune sera react most strongly with the homologous organ extract, a weaker reaction takes place also with the other organ suspension. This is probably due at least partly to the presence of common organismal (species) differentials in brain and epiphysis, as is indicated likewise by the fact that the immune sera against unheated beef brain and epiphysis suspensions react also with beef serum.

7. Fleisher showed that in liver and kidney there exist species-specific as well as organ-specific substances; in addition to strictly organ-specific substances there are others which are similar to or identical with substances present in certain other organs. He used the complement fixation test, but before making the latter he determined the presence of specific and other not strictly specific substances in the immune sera by means of absorption. Also, according to Witebsky, immunization with suspensions of kidney and liver leads as a rule to the production of both species- and organ-specific antibodies, but the former predominate. It is also of interest that precipitins and hemolysins do not develop as readily after injection of these organ suspensions as after injections of serum proteins and erythrocytes; they are found only in some of the immune sera against these suspensions.

In recent experiments Henle and Chambers showed that if rabbits are immunized with particles 0.1-0.3 micra in diameter, obtained through centrifugation of various organ suspensions of the mouse, organ-specific agglutinins can be obtained from brain, liver, kidney and testicle; negative or doubtful results were obtained from muscle, lung, pancreas and spleen. Liver and brain particles from ferret reacted likewise in an organ-specific manner with the corresponding anti-mouse sera, whereas ferret kidney and muscle particles behaved differently. These experiments indicate therefore a very marked organ-specificity of several organs of the mouse, which may have been associated with a species specificity. These particles consisted of nucleoproteins as well as other extractable substances (lipids). Claude had formerly shown that similar particles behave tinctorially and chemically like mitochondria. It is therefore possible that in these experiments mitochondria were the substratum which yielded the organ-specific reaction, but it is more probable that these particles corresponded to the "particulates" which Bensley described in the liver of guinea pigs; these particulates are similar in their chemical constitution to mitochondria, but they are smaller in size.

8. In this connection we may also refer again to the experiments of Mann and Welker, who found that it is possible to produce in rabbits precipitins for the proteins of human and rat carcinoma. The immune sera against human cancer reacted with autolysates of human cancer, but not with those of rat cancer, and the immune sera against rat cancer reacted with autolysates of rat cancer, but not with those of human cancer. There was some indication that the number of positive results was greater if the cancerous tissue,

the fact that in the egg yolk reserve substances are involved, in which species differences have developed apparently independently of the general organismal differentials. A similar reservation should, perhaps, be made also in regard to the other instances cited by us, in which organismal differentials seemed to be associated with the organ differentials. The possibility cannot be excluded that there are present in various organs, species-specific substances which, to a certain extent, are graded according to relationship, but which need not be identical with the ordinary organismal differentials. Just as the structures of organs show certain gradations which agree with relationships, so there may perhaps be present in these organs substances and also structures of a particular kind corresponding to this specificity. They would represent secondary or accessory organismal differentials; while the organismal differentials, which can be recognized by transplantation and especially by the cellular reactions against the transplants, would be the basic, primary organismal differentials. Inasmuch as it is not possible in many cases to apply transplantation tests for the differentiation of these types of organismal differentials, it must be stated again that the term "organismal differentials" is used here, and also in some other chapters, in a more general sense, as representing substances which are graded in accordance with the phylogenetic relationship of the organisms from which they are derived, and that there are among these organismal differentials, in the wider sense, the primary organismal differentials, which are characterized by their presence in all the tissues and organs of an organism; the most characteristic constituent of the latter type of organismal differentials is the individuality differential, which occurs in all or almost all of the tissues and organs of an individual, and which differentiates the individual from all the other individuals of the same species.

In the case of the proteins of the egg white, Hektoen and Cole have shown that of the five proteins of the white of hen's egg, four are quite distinct from the proteins of chicken plasma and only the conalbumin of the egg seems to be identical with serum albumin. A common-immune reaction between egg white and blood plasma of chicken depends therefore upon the admixture of a protein which is identical in both. On the other hand, there exists a pronounced species-specificity of the egg-albumins of various species, and the crystallized egg-albumins of such nearly related species as chicken and duck are immunologically not identical (Dakin and Dale). Of course, in the egg yolk and egg white we have to deal not with substances representing the embryonal precursors of the organismal differentials, but with auxiliary substances surrounding the embryo or serving as food for it. It might therefore be expected that they are chemically and immunologically distinct from the essential constituents of the developing or adult organisms; they represent paraplastic substances which are formed in the adult animal.

We see then, that as a rule substances or cells which contain organ differentials also contain organismal differentials, but that the proportion in which these two differentials are present in the same substance or cell differs in different cases. It seems furthermore that in certain instances organ and

Similar are the conditions in fishes, as Kodama has shown. Immune sera against fish spermatozoa are organ-specific; they react with spermatozoa of their own as well as of related species, but not with the extract of fish muscle. However, they are directed also against organismal differentials or their precursors, as is shown by the fact that they may respond in a quantitatively graded manner to the spermatozoa of different species of fishes, in accordance with the phylogenetic relationship of the latter.

The investigations on antisperm immune sera present an interesting problem, inasmuch as in this case we may have to deal with antigens which are constituents not of somatic cells and tissues, but of germ cells from which the somatic tissues develop, after sperm and egg have united during the process of fertilization. These germ cells must, then, possess substances, which behave like species differentials, as well as the precursors of substances, which distinguish different individuals. They must, in addition, contain substances which are specific for this type of cells and which correspond therefore to organ differentials. But these differentials would not be identical with the fully developed substances present in the adult somatic tissues; in general, the specific substances present in the germ cells would represent, rather, the precursor differentials, from which the substances in the adult organism develop. This follows from what is known of the mechanism of embryonal development and from investigations on the lens of the eye and the brain, which indicate that also the antigenic function of the organ differentials arises only in the course of embryonal life. However, the possibility exists that, after all, the immune sera against spermatozoa do not develop in response to antigens contained in the spermatozoa proper, but to a constituent of the spermatic fluid; still even then these antigenic constituents would presumably not be derived entirely from ordinary somatic tissues, but also from constituent parts of the spermatozoa or of the cells from which the latter develop. This is suggested by the fact that also autogenous substances may in this instance have antigenic power.

10. It is possible to immunize rabbits against the yolk of the chicken egg. The antiserum thus produced forms specific precipitates with the egg yolk of fowl, but not with their blood serum or with chicken embryo extract. We have to deal, here, with organ-specific substances. The reaction is strongest with the egg yolk from fowl, while a weaker reaction takes place with the yolk from other birds, but not with the yolk of fish or reptile eggs (Seng). There exists in this case, therefore, a certain quantitative gradation which corresponds to the systematic relationship of the organisms involved; but this correspondence is not complete, inasmuch as the intensity of the reactions among different species of birds does not seem parallel to their relationship; and, furthermore, the reactions do not agree with the serum precipitin reactions. There is, then, present in the yolks of eggs a system of substances which differ among themselves in their structure in a manner which corresponds, to some extent, to the differences in systematic relationship; yet these relationship differentials in the egg yolk appear to be independent of the organismal differentials of the serum proteins. This is perhaps due to

differential. Thus homoiogenous lens material does not commonly produce antibodies against lens, but Hektoen and Schulhof found that it may do so if the rabbit which is to be immunized by means of homoiogenous lens, has on a previous occasion been sensitized against heterogenous lens. Otherwise, if homoiogenous lens does elicit formation of immune substances, these are very weak; this seems to be true also of spleen. Similarly, it is as a rule necessary to use heterogenous brain in order to produce organ-specific antibodies against this tissue; a heterogenous carrier must be combined with alcohol extracts of lens or brain to produce immunization. On the other hand, according to Kato, rabbit fibrinogen may elicit in rabbits which are injected with it, the formation of antibodies against this antigen, although it possesses the same organismal differential.

Likewise in the case of organ globulins, including thyreoglobulin, homoiogenous immunization seems to succeed, perhaps because these globulins do not occur normally in a free state in the various organs but are bound to other substances, and if they are freed from the latter, they are strange to the organism which is not adapted to their effects. In the case of spermatozoa, it seems that even autogenous cells may serve as antigen, and the same has been claimed for the lens of the eye and for the skin by some authors, but this has been contradicted by the findings of others. It is conceivable that organ differentials may perhaps act as autogenous antigens under certain conditions, although the organismal differentials cannot act as such. The organism and all its parts are adapted to the autogenous organismal differential because it is present in all, or almost all, the organs of the body, whereas each organ differential is limited to a certain restricted area and is therefore strange to other areas. There may be also some other variations in the reactions of different organ differentials. Thus if once the antiserum has been produced, it may react in vitro even with antigens of a homoiogenous nature, at least in the case of lens and brain; but as far as antiserum against fibrinogen is concerned, a reaction seems to take place more intensely with heterogenous than with homoiogenous substances.

In regard to the chemical character of the organ differentials, it appears that different types of substances may be involved. We have seen that thyreoglobulin as well as globulins from other organs may serve as organ-specific antigens. Similarly, fibrinogen, serum-globulin and hemoglobin possess a substance (or organ) specificity in addition to species specificity. In the lens Hektoen and Schulhof have shown that the two crystallins, which are of protein character, as well as the whole lens can serve as organ-specific antigens. In brain and epiphysis organ differentials, which also are presumably proteins, have been demonstrated. We may then conclude on the basis of these immunological findings that organ differentials may be of a protein nature. But there are some other data which indicate that also substances of a different kind may thus function. In lens, brain, carcinomatous tissue, and also in leucocytes, substances which seem to represent organ differentials can be obtained by means of alcohol extraction. Such alcohol soluble extracts may react in a specific manner directly with the antibodies,

organismal differentials can give rise to distinct antibodies, which may be separated by means of specific absorption. This relationship between organ and organismal differentials is further confirmed by a study of the origin of the organ differentials during embryonal development. Von Szily has shown that human fetal lens does not yet possess the marked organ-specificity which is displayed by the adult lens, and that, correspondingly, the anti-human fetal lens serum reacts also with human serum-albumin. Hektoen and Schulhof confirmed this finding, although they did not observe it as regularly as von Szily. In addition, the immune serum against fetal lens shows a greater affinity for lens material from the same species than for that from a more distant species; both of these reactions, indicating the presence of organismal differentials in the fetal lens, may be lost in the course of further differentiation of the lens tissue. We may then conclude that in the fetal lens, which differs also structurally from the adult lens, organismal differentials are more and organ differentials less pronounced than in the case of the adult lens, and that as the result of complete structural differentiation the significance of the organismal differentials diminishes, while that of the organ differentials increases. Similarly, Witebsky finds that the organ-specific lipid constituent of the brain appears only when a certain stage of embryonal development has been reached, and that it is not yet present in the brain of very young embroys.

During embryonal development, it may be assumed, we have at first to deal with substances in which the organismal differentials are prominent, but in the course of further embryonal development changes tending toward greater differentiation of the parenchyma and toward the formation of paraplastic substances take place, which are specific for a particular organ, and concomitantly with the increase in organ specificity the organismal specificity decreases or may be lost almost entirely, at least as far as serological tests indicate. The substances endowed with a marked organ specificity are formed therefore from substances which possess a greater organismal specificity. With the increasing complexity of an organism, not only the organismal differentials become more refined—as is indicated by the transplantation method—but also the organ specificity becomes more pronounced. An analogous process takes place continuously in certain tissues during adult life. Certain cells in which the organismal differentials are in all probability as yet preponderating, become transformed into material in which these differentials decrease in importance or are lost altogether, and in which correspondingly the organ differentials begin to predominate. Such a process seems to occur during the transformation of epidermal cells into keratin, and presumably also during other tissue differentiations, and this change in the differentials is apparently a characteristic feature of tissue differentiation in general.

Organ differentials occur then, ordinarily, in combination with organismal differentials. In order to immunize against an organ differential of a non-protein nature it is usually necessary to employ protein substances which possess different species differentials and which act as carriers for the organ

these species. They may call forth the production of a number of different immune substances, which differ from one another by the possession of different species differentials, and the antibodies corresponding to the species differential of the antigen used for immunization predominate over the associated antibodies in their combining power with the antigen.

The experiments with egg yolk suggest that certain substances which serve as reserve material, or are of a paraplastic nature, may undergo in the course of evolution chemical changes which more or less correspond to the systematic relationship of the species in which these substances are found, but that these chemical transformations may be independent of and may follow a somewhat different course from those which concern the primary, typical organismal differentials.

as, for instance, those directed against leucocytes, brain or carcinoma; on the other hand, they serve as organ-specific antigens in combination with heterogenous sera functioning as carriers, as has been shown in the case of lens and brain.

As far as the immune serum against leucocytes is concerned, the reaction with the organ-specific component of these cells seems to be intensified if the alcohol soluble fraction is used as antigen, but the species-specific constituent is also present in this alcohol soluble fraction. Still more pronounced organ-specific effects can be obtained, according to Witebsky, if boiled suspensions of leucocytes serve as antigens. In such immune sera the organ-specific component predominates decidedly over the organismal-specific component, which latter may be lacking altogether. Likewise, through heating of thyreoglobulins the species-specific differential can be destroyed, while the substance- and organ-specific component remains preserved. In some instances the alcohol soluble organ differentials were found to be the more specific ones.

We may then conclude that organ or substance specificity may be associated with an active protein, or with a different substance which in combination with a protein serves as antigen. In the case of thyreoglobulin or other organ globulins, the organ specificity is presumably due to a sidechain attached to a protein. This sidechain may act similarly to the radicles introduced into complex proteins, or, in general, into complex colloidal substances in the experiments of Obermayer and Pick, and of Landsteiner and his collaborators. Graded reactions between different organs may perhaps depend upon a multiplicity of differential substances, some of which may be common to them while others are distinctive for certain of these organs. On the other hand, the organismal differentials are native proteins and they are therefore destroyed by heating, in contrast with the organ differentials, which are not destroyed by this procedure. There is however, some evidence that in some organismal differentials an alcohol soluble component may be present; in this case we may have to deal with the secondary or accessory type of organismal differentials.

Absorption experiments have shown that after immunization with apparently single substances, such as fibrinogen and thyreoglobulin, antibodies develop not only against the fibrinogen and thyreoglobulin of the species which served as antigen—these represent the principal antibodies—but also against the corresponding substances of related species; these would be associated antibodies. Now it is possible, as especially Hektoen and his collaborators have shown, to remove all the antibodies against certain substances, the principal as well as the associated ones, by absorption with the antigen from the original species, while only the associated, but not the principal, antibodies are removed by absorption with the differential substance derived from related species. Similar observations have been made by various investigators also in the case of other antigens and antibodies. Here apparently are involved single substances calling forth the production of antibodies, and we must therefore assume that in the molecules of these substances graded differences exist in different species, which correspond to the relationship of

quently the hypersensitiveness may extend to other tissues. As stated above, under some circumstances these localized reactions may appear negligible as compared with the general reactions which take place; this occurs, for instance, when a large quantity of the offending substance, such as a foreign serum, enters into the circulation.

The condition with which we have to deal in idiosyncrasy is evidently very similar to that observed in experimental anaphylaxis produced in animals by repeated injections of substances of a protein character. Opie has made it very probable that local anaphylactic reactions, as those characteristic of the Arthus phenomenon, are due to a local interaction between the antigenic protein and the precipitin which developed in response to the antigenic protein. The presence and significance of precipitins in this reaction has recently been confirmed by Cannon, who used more accurate quantitative methods for the determination of circulating precipitin and thus demonstrated the parallelism between the amount of precipitin formed and the strength of the allergic reaction. However, at present the possibility cannot as yet be entirely excluded that also other types of antibodies may be involved in anaphylactic phenomena. Anaphylactic shock corresponds to the general reactions noted in some cases of serum disease; the local status of anaphylaxis, either in the skin as seen in the Arthus phenomenon, or in the intestines (Schultz), uterus (Dale), or blood vessels (Friedberger), corresponds to the types of local hypersensitiveness as they become manifest in various cases of idiosyncrasy. However, the anaphylactic shock of the guinea pig, which is the animal most commonly used in the study of this condition, depends mainly upon a localized hypersensitiveness of the bronchial musculature; but there may also be associated changes in the nervous and circulatory systems. If we except some minor variations, there are two main differences which have led to a separation of the state of idiosyncrasy from that of anaphylaxis: (1) While in anaphylaxis the abnormal reaction indicating hypersensitiveness can be traced to a previous sensitization by the same substance which subsequently elicits the reaction, in idiosyncrasy the reaction may be induced by a substance with which the body has apparently not previously been in contact; (2) while in anaphylaxis we have to deal with a hypersensitiveness to protein substances, in the case of idiosyncrasy the active substance may be of a much simpler character. But in some instances of idiosyncrasy the chemical character of the active substance is unknown, and it is possible that we may have also, in idiosyncrasy, sometimes to deal with protein substances. (3) In general, anaphylaxis is a well defined condition of hypersensitiveness which may be experimentally produced in animals, idiosyncrasy is a condition of hypersensitiveness which apparently occurs spontaneously in man. In regard to the first of the differences between these two states mentioned, there is much evidence of a clinical as well as of an experimental character, which suggests that also in idiosyncrasy in man a previous but unsuspected sensitization may often have taken place. In some of these instances the sensitization may even have occurred during intrauterine life by way of the placenta, in others it may become manifest only a considerable time after contact of the

Chapter 7

Idiosyncrasy and Anaphylaxis and Their Relation to Organismal Differentials

THE TERM "idiosyncrasy" implies a peculiar state of hypersensitiveness to a certain substance, which may characterize an individual and distinguish him from others. In an analysis of individuality a discussion of such a condition should therefore be of interest. While usually only a small minority of persons are affected by an idiosyncrasy towards a substance, after all, the frequency with which various substances are responsible for such a condition differs greatly. There exists, for instance, a potential idiosyncrasy to the injection of foreign serum, especially horse serum, causing serum disease among a considerable number of individuals; likewise, the tendency to become hypersensitive to extracts of ascaris is almost universal among those infested with this parasitic worm. On the other hand, a hypersensitiveness to chicken egg is not frequent, while to less complex chemical substances, such as antipyrin, it is quite rare.

However, idiosyncrasy has an additional meaning. It signifies an individual state, which is not explained solely by the specific character of the substance eliciting it, but which, to a large extent, is due to the characteristics of the individual affected. Certain of the principal mechanisms involved are now understood, at least in their general outline, but others are as yet unexplained.

As a rule, hypersensitiveness to most of the substances with which we have to deal in indiosyncrasy is localized in definite tissues, without otherwise affecting seriously vital functions of the organism; but in certain cases central mechanisms, on the integrity of which all other functions depend, may be involved and then an idiosyncrasy may cause rapid death. Such an effect may be observed, for instance, in the so-called serum disease, where in some individuals even a first injection of a heterogenous serum, usually horse serum, may call forth very acute general reactions not unlike those of anaphylactic shock. The organs most commonly affected in idiosyncrasy are the respiratory system, especially the nasal mucosa in hay fever and the bronchi in asthma, the gastro-intestinal tract in food hypersensitiveness, the skin in many conditions in which certain substances act primarily on this organ; and the skin may show reactions also in cases in which primarily other organ-systems are involved.

While thus one idiosyncratic individual may differ from another one as to the factor which causes the hypersensitiveness and elicits the abnormal reactions, the modes of reaction and the organ-systems which are hypersensitive are remarkably similar in different individuals. In general, it seems that the tissue on which a given injurious substance acts primarily, is the one which becomes primarily hypersensitive to that substance, although subse-

indicates that immune bodies may develop under conditions in which the action of definite antigens can be excluded.

It has been observed that an individual who has manifested signs of hypersensitiveness in one organ or tissue is apt to become hypersensitive also in another organ, and perhaps to another agent. As already stated, it is largely the place where a substance acts on the body which determines the tissue that will become hypersensitive to a given substance in an individual, and which furthermore determines the character of the symptoms which will develop; the recipient tissue is the one which, as a rule, tends to become hypersensitive. There is, in addition, a specific tendency in some individuals to become sensitive to certain substances, as for instance, poison ivy. Furthermore, we cannot exclude the possibility that in certain individuals there may be a greater tendency of a special organ or tissue to be affected, while other tissues are exempt, and lastly, while, as stated, the set of symptoms in a particular tissue or organ-system is usually very similar in different individuals exhibiting idiosyncrasy, irrespective of the agent which has caused the hypersensitiveness, nevertheless, minor differences seem to exist; for example, some agents more than others tend to lead to the production of eczema of the skin.

In discussing the similarities and differences which exist between the experimental state of anaphylaxis in animals and idiosyncrasy in man, we have referred to the fact that simple chemical substances, which cannot themselves elicit experimental immunity or anaphylaxis in animals and thus cannot serve as antigens, may induce idiosyncrasy, or, to use a term introduced by Coca, may act as atopens, against which an idiosyncrasy may develop. However, this difference between anaphylaxis and idiosyncrasy has lost much in significance since Landsteiner has shown that relatively simple chemical substances (haptens) may serve as antigens if they are combined with foreign sera serving as carriers. But there still remains a definite quantitative difference between the substances which serve as atopens ("idiosyncratogens") and the substances used by Landsteiner. The latter were organic substances, which were rendered more complex by the introduction of certain groups, as, for instance, the azo group, or they were organic dyes in combination with tyrosin and resorcin, while the former may be very simple inorganic substances. However, as first shown by Obermayer and Pick, also relatively simple inorganic groups like iodine may determine a new specificity if introduced into serum protein. There exists a further similarity between certain experimental findings of Landsteiner and observations which have been made in idiosyncrasy. Landsteiner has shown that different stereoisomers may give rise to specific states of anaphylaxis and also that the ortho, para, and meta positions, respectively, of certain groups in the molecule may determine specificities. Correspondingly, Nathan and Stern observed in a person an idiosyncrasy for meta-dihydroxybenzene, although there was no reaction to ortho- or para-dihydroxybenzene.

It is possible to accomplish a passive transfer of the state of anaphylaxis from one animal to another by injecting blood serum of the anaphylactic

individual with a certain substance had taken place; in still other cases it appears to develop only gradually during the continued action of an ageot which has been introduced into the bodyfluids. However, in every instance among a number of individuals treated apparently in the same manner, only certain ones manifest such signs of hypersensitiveness; and the persons thus affected may be few or many under varying conditions.

It has been shown especially by Cooke and Van der Veer that the tendency to become sensitized against a given agent is often a hereditary characteristic, in which apparently Mendelian ratios can be demonstrated. The stronger the hereditary tendency is in children, the earlier the hypersensitiveness to foreign protein appears. Thus, if both parents transmit to the child the tendency to hypersensitiveness, the idiosyncrasy tends to appear, on the average, earlier than when the transmission is unilateral. In the case of Primula extract and nickel salts, Bloch has shown that while some persons can be more readily sensitized than others, all persons can, in the end, be made experimentally hypersensitive to these substances. But to other substances, such as iodoform, salvarsan and mercury, it is much more difficult to obtain a hypersensitiveness. The conclusion may then be drawn that there may exist a hereditary predisposition which determines the readiness with which an individual can be sensitized against a certain substance.

There enter, thus, two separate factors in this set of phenomena: (1) A hereditary tendency to become more or less readily sensitized by contact with a certain substance; this is a factor which seems to act in a quantitatively graded manner; (2) a sensitization which takes place as the result of contact with a certain substance. The greater the predisposition is, the more readily is the sensitization accomplished. We have here evidently to deal with conditions similar to those which have been noted in a number of other pathological conditions and especially also in cancer. In the latter condition we expressed the relation between the inducing factors and the disease by the formula H (Heredity) $\times$ S (Stimulation) $=$ C (Cancer). Similarly in idiosyncrasy, the relation apparently exists: H (Hereditary predisposition) $\times$ S (Sensitization) $=$ I (Idiosyncrasy). In malignant tumors we find all degrees of hereditary predisposition to cancer, and in some cases cancer may develop apparently spontaneously without long stimulation of tissues, as, especially, when certin embryonal abnormalities end in cancer formation. Perhaps also in cases of idiosyncrasy in which the quantity of predisposition exceeds a certain limit, the quantity of external factors needed for the establishment of this condition becomes so slight that these may escape recognition. Therefore, in some instances the idiosyncrasy may become manifest apparently on a first contact with a given substance. It is possible that we have to deal here with a condition similar to that noticed in those diseases caused by microorganisms or viruses, where, in some individuals, there seem to occur spontaneously formed antibodies against the causative factor. In this latter instance, also, the question arose as to whether or not these antibodies owed their origin to the action of an antigen, which might perhaps not be identical with the agent causing the disease. However, there is some evidence which

primarily with a hypersusceptibility of epidermal cells rather than of blood vessels. On the other hand, it is the latter which are essentially affected in cases in which urticaria develops rapidly following the application of a substance towards which a person shows an idiosyncrasy.

A number of years ago the writer investigated this question as to whether a piece of uterus, which has been sensitized to horse serum through a previous injection of this substance into a guinea pig, would elicit a more rapid and a more intense lymphocytic infiltration than a non-sensitized piece of uterus after homoiotransplantation into a non-sensitized guinea pig, when the latter was injected with horse serum following transplantation of the piece of tissue. The result was negative; the response of the host to the transplanted piece was not altered. It seems, then, that the chemical change underlying hypersensitiveness does not increase the reaction characteristic of homoiotransplanation. Somewhat similar are the recent observations of Aronson, who found that if a guinea pig is made hypersensitive to horse serum, the spleen and bone marrow of this animal have not thereby become particularly sensitive to the effects of horse serum if the latter is added in vitro, although the injection of horse serum into the skin of the intact animal would elicit the Arthus phenomenon. On the other hand, if a guinea pig has been infected with tubercle bacilli, its tissues are readily injured through addition of tuberculin, either in vitro (Rich and Lewis; Aronson), or after homoiotransplantation (Pagel).

So far, we have referred to substances strange to the body as exciting factors in the production of idiosyncrasy. Is it possible that also autogenous substances, those originating in the same individual, may cause sensitization? Observations indicating such an occurrence are on record. Thus Duke found that in several cases, in which, following pregnancy, milk was retained in the breast or in which lactation was much prolonged, a state of hypersensitiveness to the autogenous milk developed. Injection of the patient's milk into the skin not only gave rise to local skin reactions, but also to asthmatic attacks. Furthermore, the hypersensitiveness could be transferred by the Prausnitz-Küstner method to other normal persons, but it was only human milk, and not cow's milk, which elicited these reactions, indicating that in all probability an organismal (species) differential was involved in this condition. Milk does not, under normal conditions, circulate in the body-fluids and is, therefore, strange to the central organ-systems of the body; hence the occurrence of an autogenous sensitization is understandable under such circumstances. Furthermore, there is reason for assuming that here an inherited predisposition to such a sensitization may play a certain role. In this connection we may again refer to the experiments of Guyer, who believes that in the rabbit precipitating antisera can be formed against lens substance through injury to the animal's own lens, and to the corresponding experiments of Henshaw, who found that it is possible in the guinea pig to produce sensitizing antibodies of an autogenous nature by the application of ultra-violet radiation to the skin.

As already mentioned, non-protein substances in combination with serum

animal into a normal one. While there is reason for assuming that it is the "sessile" antibodies, those localized in certain cells and tissues, which are responsible for the anaphylactic reaction taking place in contact with the antigen, at the same time these antibodies *may also be circulating in the blood* and can then be transferred to another individual; in the latter these specific antibodies again may attach themselves to certain cells and tissues and render them hypersensitive to the action of the specific antigens. Similarly, in idiosyncrasy it is possible to transfer this condition passively in many, although not in all cases, by injecting a small amount of blood serum of an idiosyncratic person intracutaneously into a normal one. If on the following day the antigen is re-injected into the same place of the skin, a marked reaction, indicating hypersensitiveness of the treated tissue, appears. This is the Prausnitz-Küstner reaction. This reaction is positive especially in the transfer to other persons of human blood serum from individuals hypersensitive to plant pollen, egg white, cow's milk, fish, or horse dander. On the other hand, injection of the serum from cases of drug idiosyncrasy does not lead to passive transfer of the hypersensitive state. The blood serum of the hypersensitive donor, in whom the Prausnitz-Küstner reaction is positive, contains an antibody, reagin, which can combine with the tissue of a normal person, into whom it has been introduced, and make this tissue hypersensitive. Such serum, or rather the antibody which it contains, may also bind complement when mixed with the specific antigen (allergen), or it may neutralize the latter. It has been possible to accomplish passive transfer of such antibodies also in the guinea pig. For this purpose it is necessary to inject larger quantities of the blood serum; the antibodies again become sessile in certain instances and induce hypersensitiveness. However, the number of cases of idiosyncrasy in which this last named procedure has succeeded is much smaller than that in which a transfer from man to man could be accomplished by the Prausnitz-Küstner method. It seems that the passive transfer of idiosyncrasy succeeds better if the serum containing the antibody is obtained from animals nearly related to those which are to be passively sensitized. On the other hand, if the serum which serves as carrier comes from a more distant species, then it is liable to elicit the production of neutralizing immune substances in the injected animal.

There is an additional method which allows the passive transfer of hypersensitiveness, although in a much more restricted sense. It has been shown by Naegeli, de Quervain and Stalden, in a case of hypersusceptibility of the skin to antipyrin, in which the skin was sensitized not throughout the body but only in certain areas, that following autotransplanatation of a piece of the hypersensitive skin to a place where the skin was normal, the transplant retained its hypersensitiveness in the new situation. It may be concluded, therefore, that the hypersensitiveness actually resides in the tissues, and in vitro experiments in which the skin was exposed to the influence of antipyrin, it could be shown that it was the epidermal cells in which the specific changes had taken place; these responded to contact with this substances with solution processes. We have, therefore, to deal in instances such as this,

primarily with a hypersusceptibility of epidermal cells rather than of blood vessels. On the other hand, it is the latter which are essentially affected in cases in which urticaria develops rapidly following the application of a substance towards which a person shows an idiosyncrasy.

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proteins, may serve as antigens and call forth anaphylactic states. Landsteiner employed for this purpose heterogenous serum and observed that the same hapten in combination with a heterogenous serum, different from that which was used for sensitization, may react with the antibody; under certain conditions even the hapten, as such, may be able to give rise to this reaction. According to Klopstock and Selters, it is possible to sensitize guinea pigs against diazotized atoxyl by intravenous injection of a combination of this atoxyl preparation and guinea pig serum. However, in order to accomplish a sensitization by means of subcutaneous application of the antigen it is sufficient to inject diazotized atoxyl alone, without the combination with guinea pig serum. A reaction indicating hypersensitiveness is elicited in the sensitized guinea pig by the intravenous injection of diazotized atoxyl and guinea pig serum; but again, a reaction can also be obtained by subcutaneous injection of the atoxyl preparation alone, without the combination with guinea pig serum. In the latter case the reaction consists in a localized necrosis, a condition closely resembling the Arthus phenomenon. The authors assume that after subcutaneous injection of the atoxyl preparation, the sensitized animal's own serum combines with the atoxyl and acts as carrier. In the case of sensitization with simple chemical substances, such as certain drugs and the extract of primula, it is likewise possible that a combination of the hapten with autogenous serum takes place during the process of sensitization, and also preceding the idiosyncratic reaction. But as far as we are aware, a direct proof that autogenous serum may serve as carrier in the process of sensitization to such antigens has not yet been given.

Experiments of Landsteiner and Chase showed especially clearly the essential similarity between the conditions of hypersensitivity and anaphylaxis; these conditions differ in regard to the site of the body which reacts in these states and in the greater difficulty with which the reactions of idiosyncrasy become manifest. Both are antibody reactions. Thus, intraperitoneal injections of stromata of guinea pig erythrocytes, conjugated with picric acrid or with dinitrofluorobenzene preceded by injections of dead tubercle bacilli called forth both states. Subsequent applications of picric acid and blood serum mixtures induced general anaphylactic reactions, as well as local skin responses. Instead of sensitizing with picric acid-erythrocyte stromata it was possible also to cause sensitization by injections of picric acid-guinea pig serum; however, picric acid and horse serum combinations produced only a state of anaphylactic sensitization but not one of skin hypersensitivity; to produce the latter, it was necessary to use homoiogenous serum. These experiments make it very probable that antibodies are involved in both anaphylaxis and skin hypersensitiveness. In such processes of sensitization, hereditary factors determining degrees of response of the individuals may enter (Chase); this would be in accordance with the findings of Lewis, Lurie and Webster, which have established hereditary differences between the susceptibility to various bacterial and virus infections of different strains of animals within the same species.

Though all the facts known so far point to the conclusion that idiosyncrasy and anaphylaxis are closely related or identical phenomena, certain minor

differences may perhaps exist between them. Thus, while the typical immune bodies are supposed to be serum globulins and are therefore not diffusible through collodion membranes, it has been maintained that the antibodies, which make possible the Prausnitz-Küstner reaction in serum disease and other kinds of idiosyncrasy, are dialysable.

As to the relationship between anaphylaxis, immunization and organismal differentials, states of immunization as well as of anaphylaxis may be elicited by substances carrying organismal differentials, organ differentials, and, besides, by substances which have no relation to either. These various substances may also induce a reaction in the sensitized animal. Phenomena of immunity can, moreover, be elicited against individuality differentials, but it is not yet certain that anaphylaxis to organismal differentials, which are so nearly related to those of the host, has been observed.

As far as idiosyncrasy or so-called allergy is concerned, in many instances this condition seems to be directed against a species differential. Thus, in idiosyncrasy against hair of a foreign species the state of hypersensitiveness is a specific one, affecting the hair of a certain species, although overlapping reactions do occur (W. Storm van Leeuwen). The experiments of Longcope, O'Brien and Perlzweig, and those of Forster, make it probable that, on the whole, the reactions against horse dander are specific and distinct from those against horse serum, although according to Forster cross-reactions take place to a limited extent. There may, therefore, be a common species differential involved also in these reactions. We have already referred to the observations of Duke, in which hypersensitiveness to human milk was not associated with hypersensitiveness to cow's milk. In a case of experimentally produced hypersensitiveness of human skin to various kinds of serum by means of intracutaneous injection, Frei, Biberstein and Fröhlich found a similar overlapping of the reactions to that observed in the precipitin reactions. The relationship of the species used determined the specificity or lack of specificity of the reactions. However, here we have to deal with anaphylaxis rather than with idiosyncrasy in the strict meaning of this term. In anaphylaxis, precipitins may be the antibodies involved, according to Opie.

While, therefore, organismal differentials may be concerned in idiosyncrasy as the exciting agents, they do not, on the whole, play a very prominent part; on the contrary, it seems to be characteristic of idiosyncrasy that relatively simple substances, quite distinct from the complex substances possessing organismal differentials, are the principal agents; it is assumed especially in the case of drug idiosyncrasies that the person's own serum plays the role of a carrier, to which the hapten attaches itself. But this is by no means certain; it is possible that these substances may act directly on the cell protoplasm of the sensitive tissues and here call forth specific reactions.

Also, other investigations indicate the relative independence of conditions of hypersensitiveness from strange organismal differentials. Thus while in general, in immunization against organ differentials it was advisable to select as antigens sera of a heterogenous nature, which acted as carriers for the specific organ-specific haptens, in order to produce skin hypersensitiveness

homoiogenous sera seemed to be preferable as carriers; at least this was found to be the case in some experiments. It may be assumed that under these conditions the conjugation with the hapten made the serum so strange to the receptor tissue as to prevent a chemical interaction between the receptive cells and the antigen, notwithstanding the identity of the species differentials in both donor and host. We have perhaps, to deal with quantitative gradations in such cases, in the sense that if a substance does not usually enter into combination with a certain type of cells, it has a better chance to act as an efficient antigen, and only slight alterations in the chemical constitution of such a substance are required for its antigenic function, whereas substances similar to those which commonly come in contact with the cells cannot readily act as antigens. Frequent contacts of this nature may be assumed to occur between constituents of homoiogenous blood plasma and cells of spleen or bone marrow, in contrast to skin cells, where such an intimate contact does not usually take place.

We have referred to the significance of an inherited predisposition in idiosyncrasy. While a genetic basis may be conceded, the predisposition to idiosyncrasy seems not to be of a limited nature, directed against a specific agent, but rather of a more general character. According to Cooke and Van der Veer, it is determined by a single dominant factor; but until quantitative gradations in the degree of predisposition have been taken into consideration, the mode of inheritance in idiosyncrasy must be left undecided.

The anaphylactic phenomena, on the other hand, which have been studied mainly in animals, manifest pronounced species differences as to the readiness with which anaphylactic reactions can be elicited and as to the organs and tissues involved. Thus the facility with which the Arthus phenomenon can be produced varies very much, and, likewise, the relative importance of blood vessels and bronchi in anaphylactic conditions differs greatly in different species. In the rabbit, the local reactions of anaphylaxis are very marked in the skin, while the stomach responds very weakly, and the intestines not at all; in the dog the order of sensitiveness is just the reverse. In such cases, again, differentials are involved, which form a part of the Mendelian mosaic in an organism. In the predisposition to idiosyncrasy, we have presumably to deal with a character which forms a part of the mosaic constituting an individual, and, as we have seen, this is to be distinguished from the individuality differential which characterizes an individual as a whole.

The idiosyncrasies, as far as they are known to us, concern human beings and represent one of their individual characteristics. They are therefore comparable to various mosaic characteristics which may serve to distinguish individuals, such as skin patterns, scents, tissue malformations and electric brain potentials. It is an interesting phenomenon that contact with relatively simple constituents of our environments can alter the reactions of tissues with which they come in contact in such a specific and individualized manner.

Chapter 8

Toxins and Organismal Differentials

THE ORGANISMAL differentials are recognized by means of certain definite reactions which are called forth when a strange differential is introduced into an organism. The reaction may be a primary one, or it may be a secondary immune reaction. These effects may follow transplantation of pieces of tissues, as well as injections of bodyfluids or of extracts of tissues from other organisms, and they cause a disequilibrium of the host to a degree which varies with the relative strangeness of the organismal differentials of donor and host; the intensity of the reaction of the host tissues against a strange graft or material injected is to a large extent a measure of this disequilibrium and of the degree of strangeness between host and transplant, although some accessory factors may modify the intensity of this reaction within certain limits. We have found throughout that the farther distant the relationship between transplant and host, the greater the incompatability which results from transplantation. This applies to transplantation in higher, more differentiated organisms as well as in embryos of amphibia; but as a rule, it is only the transplant which suffers, the host being in such a favorable position that no serious injury is inflicted upon it in the large majority of cases. However, we have referred to some instances in which the transplant exerted a toxic effect on the host, as, for instance, in the transplantation experiments of Dürken and Kusche, and in the transplantation of amphibian eggs in the experiments of Weber and others. There were indications that in some of these transplantations we had to deal with the injurious effects of special substances rather than with the specific action of distant organismal differentials.

If these special substances orginate in an organism and normally come in contact with its various tissues acting as endorgans, and especially if they are present under ordinary circumstances in the circulating bodyfluids, they are as a rule not toxic for this organism. There is a mutual adaptation between the cells and organs and these autogenous substances. But under abnormal conditions such constituents of the body may be carried to tissues with which normally they do not come in contact, and then they may act as poisons; thus bile in contact with the pancreas or other tissues of the peritoneal cavity may be toxic, or if complex substances constituting the body, such as certain proteins, are split in an abnormal manner and these split products come in contact with organs and tissues of the organism, they may have injurious effects. Also, if hormones which are not toxins in the ordinary meaning of this term, are formed in excess, as may occur following an increase in the amount of tissue producing the hormones or following a more intensive stimulation of this tissue, or if the hormones are deficient in quantity, due to a lack of the necessary tissue in which they originate or to a lowering of its metabo-

lism, injurious effects may become noticeable and abnormal changes may take place within the economy of the organism.

But if these special substances are introduced into a strange individual they may here call forth toxic effects. In the preceding chapters we have discussed already the agglutinating and hemolytic properties of heterogenous blood sera, which seem to be conditioned by factors other than organismal differentials. We have also discussed states of hypersusceptibility in which otherwise innocuous substances become strongly toxic.

In a wider sense we may include also the strange organismal differentials among the toxins. But in a restricted sense we understand by toxins, special substances produced by certain micro-organisms or by more complex higher organisms, which are injurious for various species apparently without regard to relationship. Such toxins are substances which may be formed in special organs and they may therefore be regarded as belonging to the class of organ- or tissue-specific substances, without however representing the real organ or tissue differentials; they represent mosaic characters which have developed in addition to the typical organ differentials.

In the case of injurious substances derived from bacteria, we have to distinguish from real toxins, non-specific so-called ptomaines, which presumably are mainly split products of the medium on which the bacteria grow; the latter furnish essentially the proteolytic or lipid-splitting enzymes. The ptomaines do not therefore contain the organismal differentials. As to the exotoxins, against which antitoxins can be obtained, these are substances which are specific for certain types of bacteria; thus the tetanus toxin differs in its character and effects from the diphtheria toxin. These exotoxins apparently do not possess organismal differentials; a gradation in the character of the exotoxins produced by various bacteria, corresponding to the relationship of these microorganisms, has not so far been demonstrated. The so-called endotoxins seem to represent diverse kinds of substances, among which are nucleoproteins which may be distinctive of different species, as for instance, those obtained from pneumococci and streptococci. However, nucleoproteins obtained from various streptococci are less specific than certain other antigenic substances present in these microorganisms, and moreover, some endotoxins appear to be non-proteins and are, perhaps, glyco-lipids.

Among animal toxins it is especially the poisons found in amphibia and in reptiles which have been studied more intensively. In various species of urodeles, as well as of anuran amphibia, poisons are produced in the glands of the skin and also in the parotid gland. The distribution of these substances does not show a complete parallelism to the relationship of the various species and their pharmacological effects differ. Some apparently are identical in their action with digitalis, a plant glucoside. Thus in the European toad, Bufo vulgaris, several specific substances have been obtained from the skin and parotid gland; although in different species of Bufo such substances show some differences, essentially they are of a related nature, acting similarly to digitalis. Twitty and Johnson recently observed in embryos of Triturus [illegible] of [illegible] substance [illegible] nds of the

skin of Triturus. Embryos of related species of Triturus also produce this paralyzing toxin, but either in smaller quantities than Triturus torosus or in a weaker form. Furthermore, other types of Amblystoma are also susceptible to its action, though not to the same extent as Amblystoma tigrinum. The Triturus toxin is not poisonous for various species of Triturus. In some respects there is noticeable a relation between the amphibian organismal differentials, on the one hand, and these toxins and also the structure and metabolism of the poison-producing glands of the skin and parotid, on the other. However, in other respects these organ-specificities do not parallel the organismal differentials. Also, Bytinski-Salz has described, in the embryos of certain anuran amphibia, toxic substances somewhat similar to those which are produced in the adult cutaneous glands, but the order of toxicity in the embryonal material and in the adult skin in different species is not the same. The adult Bufo produces very toxic substances in the skin, while it is especially the embryos of Pelobates which contain poisonous material.

If we omit from consideration these specific poisons, which do not respect phylogenetic relationship as far as their orgin and their action on different organisms is concerned, there still remain substances, formed in the embryo, having toxic effects, which on the whole run parallel to the distance in relationship between the species producing the toxins and the species serving as a test object. We have, therefore, to distinguish between two kinds of toxic substances in these amphibian larvae: (1) Those which are due to peculiarities of certain organs and which apparently act more or less independently of their respective organismal differentials, and (2) those more closely related to organismal differentials, which become more severe in their effects with increasing distance in relationship of the donor and host species. But the distinction between these types is apparently not very sharp, being one more or less of degree.

In the case of snakes we find only to a limited extent, that the phylogenetic position of the respective animals bears a relationship to the character of their poison glands, the mechanism by means of which the poisons are ejected, the nature and effects of the venoms, or even to the behavior of these animals. Important distinctions which can be made between various types of snakes, as, for instance, those between poisonous and non-poisonous snakes, depend at least partly on quantitative differences in the size of the poison glands, in the amount of venom produced, and in the length of the teeth along which the venom is ejected. The Elapinae show certain characteristic features which differentiate them from the Crotalus type; thus the Cobra venoms are principally neurotoxic, while the venoms of the Viperidae exert a very strong local action. Different types of Ancistrodon show much similarity in their effects. While, however, certain characteristics are thus common to related groups of these animals, there is no definite gradation in the morphological, chemical and physiological factors which are concerned in the production and effects of the various snake venoms, corresponding to the phylogenetic relationship, and the poisons of very distant classes of animals may show marked similarities in their action. For example, the mode of action of Cobra venom is more nearly related to that of the venom of Heloderma, which

does not belong to the snakes, than to that of the venom of snakes of the Crotalus family, although the Cobra is phylogenetically far removed from Heloderma. The same lack of parallelism between the effects of the venoms and phylogenetic relationship holds good, also, as far as the susceptibility of different species of animals to these poisons is concerned. Thus in the study of the venom of Heloderma it was found that the rat and toad are relatively little susceptible to this venom; in this case we meet again with peculiarities which stand outside the system of phylogenetic relationship. Evidently we have to deal with characteristics of production and mode of action which in snake venoms and the venom of Heloderma are intimately connected with the development of certain organs. In amphibia it is the cutaneous glands and also the parotid or sublabial glands, in snakes it is presumably the parotid gland and in Heloderma the sublabial gland which undergo specific changes; within certain groups of animals the same kinds of organs may show morphologically, chemically and functionally more or less related changes.

It may then be concluded that the various animal venoms are not, in a strict sense, representatives of substances carrying the organismal differentials, but that they may have an indirect relationship to the latter in the same way as have the structure of organs and the organ differentials. Furthermore, as already stated, with certain restrictions the various substances poduced in an organism do not exert a toxic action on those cells and organs of its own body with which they normally come in contact, nor do they interact in an injurious manner with other substances normally produced in the same organism; in particular, also, they do not give origin to the formation of antibodies.

These facts apply to the animal toxins or venoms in general. The organisms in which the venoms or toxins originate are, to a large extent, although not necessarily completely, resistant to the poisonous effects they produce. Thus toads are resistant to the digitalis-like action of the bufagins and bufotoxins, but not to the bufotenins and to substances acting like epinephrin. As to the mechanism which underlies this resistance of toads, it is restricted to that organ which, in susceptible animals, is principally affected by these constituents of the venom, namely, the heart; such resistance extends also to the digitalis group of substances derived from plants. These effects must be considered as due to primary mechanisms of adaptation and not to secondary effects of auto-immunization.

Also, in reptiles the animals which are carriers of the poison glands are, to a large extent, immune against their own poisons; they possess an autogenous as well as a homoiogenous immunity. Heloderma is not susceptible to poisoning by its own venom, but it is susceptible to the effects of rattlesnake venom; likewise, certain non-poisonous snakes seem to be susceptible to the effects of Heloderma venom. However, inasmuch as the Heloderma venom is in some essential respects similar in its action to Cobra venom, it might be expected that a mutual relative immunity exists in Heloderma and Cobra for both types of venom. Such tests have not yet been made. But we have found that Calmette's Cobra antivenin exerts a certain antitoxic effect upon Heloderma venom. A species immunity to a toxin produced by

a certain species has been observed also in plants. Blakeslee noted that colchicine, an alkaloid which has specific effects on mitotic cell division, and which may induce polyploidy in plant and animal tissues treated with this substance, does not affect the mitotic divisions in Colchicum, the plant from which this alkaloid is derived; this is the only higher plan examined so far which has been found immune to it. However, Cornman has recently shown that if very large doses of colchicine are used mitoses may show the specific effect of this substance also in Colchicum; it is very probable that the relative immunity of Colchicum is due to the partial inactivation of the alkaloid produced by this plant and not to a lessened sensitiveness of the mitotic process to colchicine.

As to the mechanism on which depends the immunity of the various species against their own poisons, certain data are of interest. According to Phisalix, snakes which in general are immune to their own venom if it is administered in the usual way, are found susceptible if the venom is injected into the brain substance, thus showing that the tissue immunity does not extend to all the tissues of the animal. In this case the natural immunity of a species against its own venom is therefore not dependent upon a real lack of susceptibility to the poison on the part of those cells upon which the toxic substance principally acts. But some mechanisms presumably exist which prevent the poison from reaching the sensitive cells. In this connection it may be mentioned that Fleisher and the writer found that the liver and kidney of Heloderma, and of species related to the latter, such as the turtle, have the ability to absorb Heloderma venom more effectively than the organs of species not as nearly related to Heloderma. This suggests that these organs of Heloderma may perhaps be concerned in the natural immunity of this animal against its own venom, and that possibly proteins bearing organismal differentials may play a role in the process of absorption.

We have to distinguish from the condition of relative immunity of an organism against autogenous and homoiogenous poisons, a nonspecific increase or lessening of resistance of some species to certain poisons, irrespective of the phylogenetic relation between the species tested and the species which produces the poison. Various types of mechanisms may come into play in such species differences and they differ in different cases.

The differences in the effects which the poisons of amphibia and reptiles exert in various classes and species of animals are similar to those noted in various species of parasites and symbionts in general, and in particular, bacteria and protozoa. In neither instance are the effects determined primarily by the genetic relations between the organismal differentials of the host and of the bearer of the injurious agent, whether the latter is an animal or bacterial toxin; if the organismal differentials play a part at all under such conditions, it is only an indirect one, in the same sense in which also the effectiveness of an organ differential may be affected by its connection with an organismal differential. Thus it is evident that the virulence of certain bacteria for one vertebrate species and their lack of virulence for another does not run parallel to the relationships of the respective microorganisms and hosts.

It is well known that some bacteria are quite harmless parasites for certain mammalian species, while others are very injurious, apparently without regard to the phylogenetic relationship between microorganisms and hosts. Special mechanisms apply here which are contingent, in part at least, on the relations of these microorganisms and their toxins to specific organs. Moreover, mechanisms which differ in the case of different toxins may make a certain substance toxic for a given organ in one species and innocuous for the corresponding organ in another species. Hence it seems that the injurious effect of tetanus toxin for some species depends upon the power of the brain substance in this species to bind this toxin. In a more resistant species the brain may have a diminished affinity for this toxin. Furthermore, the degree of injuriousness of certain microorganisms, and of the substances given off by them, depends upon primarily, preformed mechanisms as well as upon secondary, acquired immune mechanisms, which latter may become effective as the result of a primary interaction between host and parasite, leading to injury in the host. The effects may also vary in very young and in adult or old host organisms.

The importance of both species and organ in determining the activity of microorganisms is especially clear in the case of certain fungi or bacteria, which function as symbionts in some species of insects. There is, here, an adaptation not only to a particular species of insects, but also to a particular receptive organ, a mycetoma, which has been formed from the fat tissue surrounding the digestive tract in this species and which is especially suitable for the life of the symbionts. These symbionts are found only in this organ and in one other location in the hosts. The mycetoma is not produced in response to the presence of the symbionts, as might have been assumed, but it develops even when they are lacking. If now the mycetoma is transplanted from the larvae of a species, such as Periplaneta or Psylla, to which these microorganisms are adapted, to the larvae of another species, for example, Tenebrio, to which they are not adapted, the transplant may remain alive throughout the life of these larvae; but such transplanted symbionts manifest no activity in their new hosts, in contrast to the activity in the old host to which they had become adapted.

The relations of microorganisms and their toxins to hosts are, then, in a general way comparable to those of poisonous reptiles, amphibia and other poisonous animals and their toxins to various species. These relations do not depend directly upon the organismal differentials of host and symbiont, parasite or toxic substance, although in certain instances phylogenetic relationships may play a limited role. The factors which determine the interaction between hosts and symbionts, parasites and toxins, are in some respects comparable to the Forssman differentials, which occur without regard for phylogenetic relationship. The relations between toxins and organism are essentially of an organ-specific character; but there may perhaps to a limited extent also organismal differentials be involved; the toxins show specific adaptations to the species in which they are produced, and there is a notable correspondence in the relations of toxins and of parasites or symbionts in general to various species acting as receptors for the toxins, or as hosts for the parasites or symbionts.

Chapter 9

The Chemical Nature of Organismal Differentials

In the preceding chapters we have analyzed by means of tissue reactions the individuality and species differentials, as well as organismal differentials in general. Immune reactions made it possible to analyze still further the species differentials and the differentials of genera, orders and classes of animals, and even of plants; but, individuality differentials were accessible to serological tests only in a very restricted way. Immune reactions can be used in the study of all those differentials which are able to function as antigens. This includes in addition to the organismal differentials, organ differentials, the heterogenetic differentials of various kinds, and the blood-group differentials, as well as specific antigens present in certain micro-organisms and metazoic cells.

In the majority of these cases we have to deal with substances which have not yet been isolated chemically, but which can be recognized and differentiated from one another by the tissue and serological reactions which they induce. As to the chemical nature of these substances, our knowledge is therefore very limited. However, there can be little doubt that the organismal differentials are proteins; this is indicated by their great sensitiveness to heat and to the action of substances which are known to denature proteins. As to the organ, heterogenetic and blood-group differentials, proteins may also enter into their constitution, but they may still retain to a certain degree at least their characteristics as antigens under conditions in which proteins are denatured. Therefore other groups than proteins form part of these antigens. They may be conjugated proteins, combinations of proteins, acting as carriers, and of complex carbohydrates, lipids, or simpler organic substances acting as haptens. The combinations with simpler substances are of significance especially in the state of hypersensitiveness. But even simple inorganic and organic substances as such, seem to be able to induce idiosyncrasy in certain individuals predisposed to this condition, although there is the possibility that even in this case they become effective only in combination with proteins. In all these instances it has been shown that as a rule the antigenic function proper, that is, the production of immune substances or antibodies, requires the combination of these non-protein substances with proteins; but if the antibodies have once been formed, they may interact in a specific manner also with the non-protein material functioning as haptens. However, it has been proven more recently by Heidelberger that in pneumococci, type-specific and species-specific complex carbohydrates are present, which may act as antigens and call forth the production of antibodies without having previously entered into combination with proteins. Specific carbohydrates have been iso-

lated also from various other microorganisms, especially from streptococci.

Recent studies of proteins make it very probable that in the organism the simpler peptid chains are present, not as such, but in association with one another, and it has been suggested that their molecular weights are multiples of a unit possessing a molecular weight of 34,500 (Svedberg). Reversible associations and dissociations may take place. According to Bergmann, such a unit is built up of 288 amino-acid residues and a protein may consist of multiples of such units. Within these units certain amino acids recur at regular intervals, which are characteristic of different proteins. X-ray studies make it probable, moreover, that such protein chains may be folded and that parallel fibers may be linked together by means of their active sidechains in definite patterns, the distance of these chains being ascertainable by the X-ray pattern (Meyer and Mark, Astbury). According to Mirsky and Pauling, these sidechains are united by hydrogen bonds between the peptid nitrogen and the oxygen of the carboxyl group.

Denaturation by heat, application of alkali, acid or various other means, is supposed primarily to bring about breaks in these sidechain bonds and to unfold the main chains. Denaturation also alters or reduces the specificity of the proteins; it may diminish or destroy the specificity of the antigens and it destroys the individuality differentials. Conversely, in accordance with this theory of protein structure, we may assume that the specificity, and in particular, also the specific character of the individuality and species differentials, depend upon the character and distribution of these patterns and linkages as well as on the chemical constitution of the amino-acids; and it may furthermore be suggested that some of these factors are specific for cell and blood proteins in different individuals; also, that all the cell proteins in the same individual must have a certain characteristic in common, which differentiates these proteins from the proteins of all other individuals. At present it seems impossible to do more than to make this general statement concerning the possible connections between the nature of the individuality differentials and theories of protein structure, of which several have been proposed.

There is a second series of investigations which may throw some light on the structure of various differentials, although they have more significance for the organ, heterogenetic and blood-group differentials than for the organismal differentials. These investigations, to which we have already referred, deal with the experimental modification of antigens and the corresponding changes in the immune substances which are elicited by the injection of the modified antigens. This method of research was inaugurated by Obermayer and Pick, who thus laid the foundation for the subsequent fargoing analysis of the chemical nature of antigens. It is of interest, in this connection, that the discovery of Jacques Loeb of the possibility of inducing heterogenous fertilization by addition of alkali to the medium in which the germ cells are suspended, and thus of modifying the specificity of the fertilization process, suggested to Obermayer and Pick the thought that also the antigen specificity might be accessible to changes by chemical means. They

therefore began to study the chemical factors underlying the species specificity of the precipitinogens. Their principal finding was as follows: The species specificity of cattle serum was not greatly altered by heat, nor by such substances as alkali, toluol and chloroform, but it was fundamentally changed by introducing the iodine of Lugol's solution into the protein molecules, by diazotizing the protein, or by producing xanthoproteins by means of nitric acid. The species specificity was destroyed by the latter processes and new specificities were created instead. The antibodies which originated through immunization with these new antigens, reacted specifically also with other proteins into which similar chemical radicles had been introduced, but no longer or very little with the unaltered proteins of the original serum. Furthermore, they made it probable that it was an aromatic constituent of the protein, tyrosin, to which the new group was anchored. They concluded, therefore, that the aromatic constituents of proteins were mainly responsible for the antigen specificity.

These observations led Obermayer and Pick to distinguish between the constitutional and the original structure of a protein; by the latter was meant its species characteristics. A first type of substances, such as acid, alkali, toluol, as well as application of heat, leave the latter intact but change the former, while introduction of a second group of radicles, such at N-N, NO_2, or J, Br, changes the species specificity. However, the distinction between these two types of specificity no longer seems to be as sharp as Obermayer and Pick assumed. A part of the species-specificity of the serum may still be left even after introduction of a new group of the second type of substances, especially after diazotation; on the other hand, specificities may be modified also by alkali and by heating. Furthermore, even the introduction of the methyl and acetyl group, or of other groups which do not combine with the aromatic constituent of the protein molecule, may likewise modify the species-specificity. Thus, as Landsteiner has shown, esterification with acid alcohol, acetylation and methylation may cause loss of specificity of a protein, although these groups do not directly affect the aromatic nucleus of the protein. But essentially, Obermayer and Pick have established some of the basic facts concerning the constitution of antigens and their species-specificity. These investigators also made the important observation that a number of partial precipitins may develop through immunization with a protein, the constitution of which has been altered experimentally; and some evidence has been found by subsequent investigators which confirms the conclusion that the aromatic protein group is of great importance for the specificity of the antigen. Thus, Wells pointed out that gelatin, which lacks the aromatic group, also lacks antigenic powers, and still later it was shown that the introduction of the metanilic acid radicle into gelatin changes the latter into a potential antigen, which reacts also with an antiserum against the combination of another protein with metanilic acid. Furthermore, Wormall found that iodine, in altering the specificity of the protein, combines with the tyrosin radicle. However, while all these data point to the conclusion that the character of

the aromatic group in the protein molecule is of great importance in fixing the species-specific nature of the latter, still it is apparently not the only determining factor.

On the foundation laid by Obermayer and Pick, Landsteiner and his associates built further and they established, among others, the following important facts:

(1) Xanthoprotein and diazotized protein show a close serological relationship; similarly, there is a strong cross-reaction between iodo- and bromoprotein; but there is a sharp serological distinction between the nitrated and diazotized protein on the one hand, and the halogenated protein on the other. These differences may depend not only on the nature and the number of the substituting groups, but also on the place of substitution, and there is an indication of gradations in these reactions. While in this way a new specificity can be produced, a remnant of the old organismal specificity may still be left, and although chemically altered horse serum calls forth the production of antibodies, which react also with other protein compounds which have been coupled with similar radicles, still the reaction may remain most intense with the substituted compounds of horse serum. Antiserum against diazobenzene serum protein from cattle, precipitates diazobenzene protein from cattle serum, but not that from human, horse or rabbit serum; nor is there a reaction with the native, unchanged serum from cattle. Thus it becomes conceivable that diseased or functionally changed tissues may give off proteins, which may act as antigens in other individuals of the same species. We have here to deal with the combination of a species and a structural specificity of certain substances, which recalls the complex specificity due to the combination of organ and organismal specificities previously discussed. However, the chemical alteration of a protein must be fargoing if the immune serum is to react with antigens derived from a different, nonrelated species into which the same group has been introduced.

(2) Introduction of complex organic groups, together with the diazo and certain other radicles, increases the specificity of the reaction to the new substance. Of special importance in determining the specificity of the azoprotein are acid groups which are introduced into the benzene ring, while the introduction of methyl, methoxyl, halogen and nitro groups is less effective in changing the specific character of the antigenic substance.

(3) Likewise, the position of certain groups introduced into the protein molecule helps to determine the specificity of the latter. Hence the same group, if introduced into the ortho, meta or para position, calls forth in each case the production of specific antibodies, although weaker cross-reactions may occur also with other than the homologous antibodies. The specificity of these substances is therefore not absolute, but relative and graded, and there are, moreover, certain preparations which elicit reactions that do not conform to the expected specificity. In addition to the ortho, meta and para positions, also differences in the stereoisomeric constitution of certain substances may yield specific antibodies.

(4) Perhaps the most important finding of Landsteiner, however, con-

cerns the possibility of synthesizing antigens by combining a substance, which alone is unable to produce antibody formation, with a foreign, heterogenous protein or serum and thus to obtain a complete antigenic substance. The serum in this case acts as "carrier" for the specific substance, the hapten, which latter does not need to be a protein. Landsteiner first synthesized in this way a hapten and a protein carrier in the case of the Forssman antigen by combining the alcohol extract from heterogenetic organs with hog serum. As in Obermayer's and Pick's observations, the protein in the original antigen and in the substance with which the antibody is tested do not need to be identical, and if they are very different, then the specificity may be limited to the hapten. The hapten alone may be able to react with the antibody, provided it possesses a sufficiently large molecule, and especially if this molecule has colloidal properties. Landsteiner succeeded by these means in separating the ability of an antigenic substance to elicit the production of an immune substance from its ability to react with such an immune substance, and he furthermore recognized as a distinct property of an antigen, or of a part of an antigen, the power to inhibit in a specific manner the reaction between antigen and immune substance. While as stated, the first of these functions requires as a rule a combination of a hapten, which may be a non-protein substance, and a carrier of a protein nature, the latter two functions may be exerted by the hapten alone.

In studying antibodies against azoproteins, Landsteiner found that the action of an antibody, which developed against a well defined chemical substance, was not confined to the antigenic substance, but it included substances chemically similar to the homologous antigen. Landsteiner concluded that the serological cross-reactions of the proteins of related animals are due to similarities in the chemical structure of these substances. This constitutes at least one of the possibilities of such cross-reactions.

As to the inhibiting effect of haptens, Landsteiner extended an early observation of Halban and thus found that even in cases in which the hapten does not undergo a visible reaction with the antibody, its presence may be recognized by its specific inhibiting effect on precipitation, complement fixation, and hemolysis, which would otherwise occur if the full antigen were brought into contact with the antibody. It could furthermore be shown that the reaction between hapten and antibody was the more specific the more complex the structure of the hapten. If more simple substances served as haptens, the reaction did not need to be specific. Again, it was especially the aromatic groups which tended to determine the specificities in the antigen-antibody reaction. By means of this reaction Wormall showed that if iodine enters in combination with the tyrosin group of the protein, it calls forth a new specificity, and that 3.5 iodotyrosin can specifically inhibit the reaction between iodoprotein and its antiserum.

We have mentioned already that the carrier protein, as a rule, should be of a heterogenous nature, but in certain cases a homoiogenous, and, perhaps, even an autogenous serum may exert a similar effect. Thus, as mentioned, Klopstock and Selters believe that in the guinea pig a combination of

the aromatic group in the protein molecule is of great importance in fixing the species-specific nature of the latter, still it is apparently not the only determining factor.

On the foundation laid by Obermayer and Pick, Landsteiner and his associates built further and they established, among others, the following important facts:

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bodies produced in rabbits by injection with the glucuronic acid antigen protects mice against infection with Type II pneumococci; the antiserum with the galacturonic acid antigen is ineffective. Furthermore, the immune serum of rabbits injected with p-aminobenzyl β cellobiuronide confers passive immunity in Types III and VIII pneumococcal infection, whereas the serum of rabbits immunized with p-aminobenzyl β gentiobiuronide is inactive. But both immune sera against cellobiuronic acid and gentiobiuronic acid provide passive immunity against Type II pneumococcus infection. If the glucuronic acids are removed from the antigens, no protection is obtained against Type II pneumococcus. It may therefore be concluded that in the latter case the two glucuronic acids are the active constituents of the antigen. On the other hand, immunity against Types III and VIII pneumococcal infection depends upon the particular union between the two constituents of the two disaccharides, cellobiuronide and gentiobiuronide, one kind of union being effective while the other is ineffective.

Not only have relatively simple organic substances, joined to protein by means of diazotization, served as haptens, but also alcohol extracts of various cells and organs which, in combination with protein, function as antigens and call forth the production of antibodies. In this way, Forssman heterogenetic antigens, blood-group antigens, especially the antigen for blood group A, and organ-specific antigens have been used. The latter have been prepared also from boiled organs. In general, these alcohol soluble haptens are heat stable. They function as complete antigens and call forth the production of antibodies in combination with protein, especially the protein of a heterogenous blood serum. At first it was assumed that the haptens in these alcohol extracts were lipids, but subsequent investigations made their lipid nature doubtful in many instances. In accordance with the work of Landsteiner and Levene, it is now assumed that the Forssman hapten is a combination of a carbohydrate and a lipid, and it is furthermore assumed that a carbohydrate may be present also in the blood-group antigen. As to the organ antigens, they may be proteins, in which, however, other groups are active than those which represent the organismal and especially the individuality differentials. The active organ differential groups are heat stable. Some organ and "substance" antigens are conjugated proteins or combinations between haptens and proteins.

In regard to the organismal differentials, especially the individuality and species differentials, these depend essentially on certain characteristic properties of proteins, which the various parts of an individual, or the individuals composing a species, have in common. In the beginning of this chapter we have already discussed some of the properties of cell and tissue proteins and have mentioned the fact that the individuality differential is lost whenever the proteins are denatured. We have furthermore stated that the process of denaturation may depend primarily on a breaking of linkages between certain sidegroups in parallel peptid chains or in the same peptid chain coiled upon itself, and such a breaking of linkages must therefore destroy the individuality differential; or it may depend upon a process of uncoiling. This does

diazotized atoxyl with the animal's own serum may serve as antigen. We have discussed this problem in a preceding chapter. However, while foreign sera seem to fulfill the function of carriers of the haptens efficiently, Armangue, Gonzales and Morata have shown that the Forssman differential, which by itself is not at all or only very slightly antigenic, can be converted into an active antigen also by mixing it with kaolin or other absorbent substances instead of with serum. Zogaya has found that a complex bacterial polysaccharide may serve as a satisfactory antigen if it is first absorbed by collodion or carbon particles. Landsteiner and Jacobs confirmed these observations, but they also noted that purified bacterial polysaccharides or other complex carbohydrates, when freed as much as possible from N-containing substances, can no longer be activated by these non-specific, absorbent colloids, and the same applies to the purified Forssman differential. It seems, then, that certain impurities which are mixed with the differentials may somehow enhance their antigenic power, and this process can be still further accentuated by combination with absorbent colloids.

It has been noted by Goebel and Avery that also some glucosides in combination with heterogenous proteins may act as haptens; in this case, stereoisomeric differences may help to determine specificity and, therefore, the substitution of a galactose for the glucose radicle in the glucoside may lead to a new specificity. The stereoisomeric differences in the galactose and glucose group resulted in the formation of specific antioodies. It is of interest in this connection that while in the composite antigen the glucoside and protein are combined into one substance, two separate antibodies seem to develop in response to the injection of this antigen, and these are apparently distinct from each other; moreover, it was found possible to remove the one by specific absorption without at the same time removing the other. The glucoside as such, acting as hapten, inhibited only the interaction of the anti-carbohydrate antibody and not that of the anti-protein antibody with the antigen. In general, it may be stated that, in accordance with the findings of Landsteiner, a simple antigen, in which the chemical constitution of the hapten is well known, may cause the production of several distinct antibodies, which are directed against different groups in these antigens and which can be removed by specific absorption. Or the antibody may represent, perhaps, a very complex composite structure, in which different groups combine with different component parts of the antigens chemically, with different degrees of firmness; and conversely, there may be different degrees of dissociation between the constituents in the antigen and antibody combination. In this way Heidelberger interpreted the occurrence of various kinds of cross-reactions between antisera and antigens.

Goebel in more recent investigations analyzed still further the conditions which cause the specificity of antigens by the use of artificial antigens against various types of pneumococci. One of these antigens contained the azobenzol glucoside of glucuronic acid, the other, that of galacturonic acid. The difference in stereoisomeric constitution of these two glucosides has a marked influence on the serological specificity of these two antigens. The immune

and much greater than the differences between the myosins of quite unrelated species.

It is probable that not only the nature of the bonds between sidechains in the same protein, but also the nature of the sidechains as such, may differ in different proteins. Likewise, according to Bergmann, the various natural proteins differ from each other in that their individual amino-acid constituents are represented by different frequencies within the complex protein molecule. This view implies that the physical-chemical and biological properties of a particular protein depend in the last analysis on the frequencies with which the constituent amino-acid residues recur within its peptid chain. However, no sharp distinction is made here between species-specific and organ- or "substance"-specific proteins. Of great interest is the suggestion of Bergmann that it is the cell enzymes, the proteinases (papainases) which not only split the proteins, but also synthesize them from the constituent amino-acids of the foodstuffs, and which, because they have a specific constitution, specifically determine the specificity of the cell proteins which they build up; therefore, the cell enzymes and cell proteins must in each instance have the same specific structural characteristics; the process of constructing these proteins would thus be autokatalytic. However, according to such a conception this autokatalytic process should primarily lead to the newformation of specific enzymes rather than of specific cell substratum. But it may be assumed that secondarily these specific proteinases would also build up the cell proteins in such a way that they possess the same characteristic species differentials as the enzymes. This conception was applied to the species differentials of cell proteins; but if it should be extended to the individuality differentials, then it would be necessary to assume that also in the different tissues of the same individual the proteinases not only possess the same species differential, but also the same individuality differential, and these enzymes should then differ in the reactions they call forth in different individuals in accordance with the differences in their individuality differentials. However, such distinctions between the enzymes of different individuals have not yet been noted.

By means of electrophoresis, Landsteiner could distinguish the egg albumins of chicken, guinea hen and turkey from those of duck and goose, but he could not establish definite differences within these two groups. It was therefore possible to distinguish between the proteins of species belonging to different orders, but not possible to distinguish between those belonging to the same order, such as chicken, guinea hen, and turkey, or duck and goose, though these could be distinguished by means of the precipitin reaction. This lack of differences in the electrophoretic mobility of some of the egg albumins obtained from different species of birds is in contrast to the discovery, by Tiselius, of three different fractions differing in their electrophoretic behavior in the apparently homogeneous globulin of rabbit serum.

It is possible that in the protein molecules the organismal differential, which may function also as antigen, is determined not by a small group, but by larger groups; this is suggested by the multiplicity of cross-reactions

not indicate, however, wherein the individuality differentials of different individuals differ from each other. In a similar manner we have seen that the introduction of new sidechains or haptens into a protein may change or destroy the species differentials; but it would not necessarily follow that actually the various species differentials represent combinations of proteins with different sidechains or haptens; on the contrary, it seems certain that as far as haptens are concerned pure proteins may be representative of species differentials. Still, our knowledge as to the chemical properties of proteins, on which these species differentials depend, is extremely fragmentary. It is the tissue and serological reactions which have given us our first basic and, so far, the only definite data regarding the organismal differentials. However, some of the chemical-physical differences which have been established between the proteins of different species may be suggestive in this connection; no positive data of this kind exist regarding individuality differentials. As mentioned, in the beginning of this chapter, more recent investigations, especially those of Svedberg, indicate that the native cell and tissue proteins are more complex and represent longer peptid chains than had been assumed. In particular, concerning the hemocyanins, Svedberg has shown that the molecular weights of these substances, as they are present in the blood of certain species, are always simple multiples of the well defined component with the lowest molecular weight. These components are interconnected by reversible dissociation-association reactions, which are influenced by the pH. The range of pH in which these complex hemocyanin molecules are stable is characteristic of the hemocyanins of different species. But marked differences in the pH stability diagram occur only for species belonging to different orders. All the species of the same order have similar diagrams. In addition to this pH range, the isoelectric point of the hemocyanins of different species is to some extent characteristic of the species.

In regard to the hemoglobins, the extensive investigations of Reichert and Brown to which we have already referred, have shown that their crystal form differs in different species; likewise, the readiness with which they crystallize differs. But there is the possibility that in these determinations other proteins from cells may have been admixed to the hemoglobin crystals and may have contributed to the differences between the crystals of different species. As to the chemical constitution of hemoglobins, it seems that the hemoglobins of horse, sheep, cattle and dog contain the same amount of the basic amino-acids, arginin, histidin and lysin, but differ in their cystin content and in the amount of total sulfur (Block and Vickery). Horse and donkey hemoglobin differ also in their solubility, although they cannot be distinguished by the precipitin test (Landsteiner and Heidelberger). Bailey has found that in myosin the amid nitrogen, expressed in percentage of total nitrogen, is about the same in mammals, birds, fish and lobster; and the same applies as far as the percentage composition of cystin, methionin, tyrosin and tryptophan is concerned. On the other hand, the differences between the amino-acids which occur in myogen and myosin within the same species are very considerable,

discussions. We have already referred to the polysaccharides which are found in cell constituents in many kinds of bacteria and which are characteristic of certain types and species of bacteria, especially of pneumococci. They were first discovered in pneumococci by Heidelberger. Each type of pneumococcus has its own kind of polysaccharide. These complex carbohydrates function, as a rule, as haptens, which in combination with foreign proteins may act as full antigens; but the polysaccharides of Types II and III pneumococci may, as such, act as antigens. As Heidelberger has shown, the polysaccharide of Type III pneumococcus consists of numerous units of cellobiuronic acid, while a single unit is antigenically ineffective, a combination of several units may unite as antigen with the specific antibody in anti-pneumococcus type III horseserum. It is possible that within the bacterial cells these polysaccharides are combined with proteins. In the case of the pneumococci it can be shown that they are not diffusely distributed within the cell, but form a constituent of the bacterial capsule. As far as a comparison is possible between simple unicellular and higher, very complex organisms, these carbohydrates may be compared to organ differentials of higher organisms rather than to organismal differentials; they are localized in certain parts of the cells and as a rule act as antigens only in combination with other substances; but at the same time they are specific for group and also for species of these unicellular organisms in the same way as organ differentials may carry a species differential. It appears probable that also in bacteria protein substances situated within the cell body are the carriers of the typical species and class differentials, and quite recently Heidelberger and Kendall have begun to separate such substances by methods which prevent or diminish much their hydrolysis during the process of preparation; some of them may be fully antigenic.

Within cells there arise also the enzymes, endoenzymes and exoenzymes, as they might be called, which likewise show various kinds of specificities. From a functional point of view, their most marked specificity relates to the substratum on which they act and which they convert into different substances, either by splitting or by synthesizing processes. Enzymes are characterized by this specific effect, by the conditions under which they act, and by their place of origin. In accordance with their intimate connection with cells, they consist of proteins which in some instances may function in combination with prosthetic groups, especially also with certain vitamins. These proteins have been obtained in crystalline form (Sumner, Northrop, Kunitz and others). It has been shown that some enzymes develop from precursor substances which also have been obtained in crystalline form (Northrop, Kunitz); thus pepsin, trypsin and chymotrypsin are derived from pepsinogen, trypsinogen and chymotrypsinogen. In addition, there has been distinguished among the pancreatic proteolytic enzymes, heterotrypsin and beta and gamma chymotrypsin. The substratum specificity of these enzymes goes farther than has been assumed, and Bergmann has shown that simple peptides can be found on which the various proteolytic enzymes of the pancreas exert a specific splitting effect. It is the enzyme itself which may convert the precursor sub-

between the organismal differential (antigen) of a certain species and the various antibodies present in the immune serum; the pattern of the protein corresponding to the order in which certain amino-acid radicles recur in the molecule may perhaps be a factor which helps to determine the character of the antibody. This view agrees also with the views recently expressed by Landsteiner and with the observations of the latter that the specificity of immune sera for polypeptides may depend upon a pentapeptide in its entirety. Therefore, large groups and their specific pattern of amino acids may determine phylogenetic relationship. On the other hand, in contrast to the organismal differentials, the specificity of other antigens such as the various agglutinogens which determine the specific blood-group reactions and which seem to be complex nitrogen-containing carbohydrates, may be quite distinct from each other and not show multiple intermediate substances. Here the differences between the various antigens can be conceived as of a more abrupt nature and perhaps due to single groups sharply differentiated from those of other analogous antigens.

While there can be no doubt that it is the proteins which primarily determine the specificity of the organismal differentials, there are some serological experiments which indicate that in certain cases also some other hapten-like substances may perhaps be concerned in similar reactions. Thus, it has been observed that while whole erythrocytes are required in order to produce species-specific antisera for the red corpuscles of certain species, alcohol extracts of the same kind of red corpuscles may react specifically with such immune sera; it appears therefore that in this case the antigen contains an alcohol soluble hapten, and as Landsteiner has shown, the hemolytic action of such species-spectific hemolysins may be inhibited by addition of ether extracts of such red corpuscles to the antibody. These observations would agree with the finding made in the course of our transplantations of tissues and previously discussed, that the species differentials differ from the individuality differentials in that the former are somewhat less heat sensitive than the latter. These complex species differentials present in erythrocytes could resemble the organ differentials which withstand boiling in contrast to the typical species differentials which are destroyed by boiling; the organ, tissue or substance specificity may perhaps reside in the hapten, while the typical species specificity resides presumably in the protein with which the hapten is associated. In these particular substances the species-specific component of the antigen may then perhaps consist of a hapten of a non-protein nature. An important point to be considered in this connection is the fact that the presence of a chemical factor; graded as to phylogenetic relationship of the animal group and characteristic of the typical organismal differentials, has apparently not been demonstrated in these antigens or in parts of the antigens contained in the alcohol extracts.

It might therefore perhaps be necessary to distinguish between the primary species differentials of protein nature, and secondary complex differentials which represent combinations of organ or "substance" differentials and the species differentials, an interpretation which we have mentioned in previous

precursor rather than in the enzyme which induces this reaction. This applies also as far as the species specificity of the enzymes and their precursors is concerned. As Herriot, Bartz and Northrop have shown, swine pepsinogen can be converted only into swine pepsin and chicken pepsinogen into chicken pepsin, irrespective of the species character of the enzyme which serves as catalyst of this reaction.

A very marked organ and perhaps also organismal specificity of enzymes has been found in Limulus (Loeb and Bodansky). In this species, urease occurs in the bodyfluid, muscle, and even in the eggs. Moreover, a urease is present in the amoebocyte tissue prepared from the amoebocytes of the bodyfluids. This enzyme has been found so far only in Limulus and not in any of the arthropods which have been examined for its presence. But in Limulus the urease obtained from amoebocytes differs from that found in the other organs or tissues in that the amoebocyte-tissue enzyme combines with various kations, and the degree of its activity depends upon the kind of kation with which it is combined; but heavy metal combinations of the enzyme are inactive, probably because they induce denaturation. This urease represents therefore, in all probability, a metal protein combination. If the enzyme obtained from various organs of Limulus is injected into the bodyfluids of this animal in sufficient quantity, urea is transformed into ammonium carbonate, a substance which is toxic and lethal.

Similar in certain respects to the action of enzymes is that of some viruses, such as the virus of tobacco mosaic disease, which has been found to be a crystalline nucleoprotein (Stanley), and bacteriophage, which is also a nucleoprotein, according to Northrop. With this interpretation accord the experiments of Bronfenbrenner and Kalmanson which have made it very probable that bacteriophages do not multiply as bacteria do, but are continually newly formed by the type of bacteria in which they originated. On the other hand there should be considered also the strong indication that bacteriophage occurs in association with larger particles of various sizes by which the phage has been adsorbed, and that the active agent represents a smaller molecule (Bronfenbrenner). Both the viruses and the phages are specific in their action as far as the character of the substratum is concerned. Thus, bacteriophages act primarily only on the bacteria in which they originated, or on nearly related microorganisms. The phages derived from different bacteria can also be distinguished by immunological methods. To a certain extent, an adaptation of bacteriophages to new hosts may take place. Likewise, bacteria and yeasts may produce new enzymes in response to altered substratum (Euler, Dubos) on which they are cultivated.

In passing from cell protoplasm to enzymes and viruses, we compare the most complex substances with other substances which are less complex and less specific. A further step leads to the hormones, some of which are still proteins, while others represent relatively simple organic substances. In different species the production and distribution of various hormones may be different, and some of the complex hormones seem to possess a certain degree of organismal-specificity (insulin, pituitary hormones), but the large

stance into active enzyme by an autokatalytic reaction. But this latter type of reaction shows only a very imperfect specificity. Pepsin transforms pepsinogen into pepsin, and trypsin causes the activation of trypsinogen, but trypsin exerts the same function also towards chymotrypsinogen. While thus the activating enzyme possesses only a limited specificity, the substratum on which the enzyme acts—in this case the precursor substance—undergoes specific changes; thus chymotrypsinogen can only be converted into chymotrypsin, whatever the nature of the activator may be.

In those enzymes which consist of a combination of a protein and a prosthetic group, specificities in the character and action of the enzymes may depend not only upon the nature of the protein but also upon differences in the prosthetic group, or in the manner in which the prosthetic group and the protein are linked. Specific differences in the production of such enzymes in different cells may, according to Robbins, depend upon the different ability of different cells to produce a vitamin which forms a constituent part of the enzyme. As to the relations between enzymes and organismal differentials, nothing is known in regard to individuality differentials in enzymes. However, there is reason for assuming that the corresponding enzymes of different species are distinct, although such differences cannot always be demonstrated by means of immune reactions. Thus Kirk and Sumner could not definitely distinguish between the urease of soy bean and of jack bean by means of the precipitin reaction or by using the protective action of immune sera as a test. But that species differences exist has been shown through a study of the solubilities of various enzymes; for example, the solubilities of cattle and swine pepsin differ from each other. In certain instances a species-specificity has been demonstrated also by the production of immune substances, especially of precipitins, and of localized anaphylaxis in the guinea pig. Ten Broeck used the uterus of the guinea pig as test organ and was able to distinguish between trypsin from cattle and swine and also between chymotrypsin and chymotrypsinogen. Seastone and Herriot, by means of the precipitin reaction, could distinguish swine, cattle and guinea pig pepsin from rabbit and chicken pepsin; but pepsin from swine, cattle and guinea pig could not be differentiated from one another by these means. On the other hand, pepsin and pepsinogen could be distinguished by the use of the precipitin reaction. Moreover, precipitins for enzymes did not react with serum proteins of the corresponding species. It may therefore be concluded that the proteolytic enzymes of the pancreas and stomach, and their precursors, possess substance and organ specificity; furthermore, that the corresponding enzymes and their precursors from different species differ in their constitution; but no proof has been given so far that this difference corresponds to the graded relationship of the various species, or that these enzymes have a chemical characteristic in common with proteins in other organs of individuals belonging to the same species; nor has it been shown that they possess individuality differentials.

We have mentioned already that in the process of transformation of the precursor substance into the active enzyme, the specificity resides in the

precursor rather than in the enzyme which induces this reaction. This applies also as far as the species specificity of the enzymes and their precursors is concerned. As Herriot, Bartz and Northrop have shown, swine pepsinogen can be converted only into swine pepsin and chicken pepsinogen into chicken pepsin, irrespective of the species character of the enzyme which serves as catalyst of this reaction.

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majority of hormones do not possess a species-specific structure and the species-specificity of their action depends on the species-specificity of the substratum on which they act. Vitamins are not of a protein nature, but they may combine with proteins to form enzymes. While, therefore, neither vitamins nor the majority of hormones possess organismal differentials, still there may be differences in the production of these substances in different species, and these differences are analogous to differences in the structure of organs and tissues and in the constitution of certain substances which occur in these organs and which are characteristic also of these species, without a relation to the phylogenetic position of the species being manifest.

To return to the starting point of this discussion: if an immune serum is produced against a conjugated protein, the immune substance in the serum may combine either with the hapten or with protein acting as carrier. In order to obtain a specific interaction of the antibody with the hapten alone, or with the hapten or substituent groups when conjugated with a carrier differing from the one which originally was a part of the antigen giving rise to the antibody, it is necessary for the antigen to contain a certain number of the substituent groups. If this number remains below the threshold of effectiveness, it is solely the carrier which induces the antibody production; we have therefore to deal here with quantitative relations (Haurowitz, Sarafran and Scherwin). In order to produce agglutinins for erythrocytes to which certain sidegroups or haptens had been attached, it is necessary for parts of the surface of these to be free of these attachments. Then the antibody will be directed also against the erythrocytes as such and not only against the hapten (Pressman, Campbell and Pauling). This condition seems to be one of the factors which determines whether antibodies other than those directed against the hapten will be able to cause the agglutination of the erythrocytes. But also the hapten, as such, may bring about an agglutination of the erythrocytes if each one of the molecules of this substance had a chance to attach to itself two erythrocytes during the process of centrifugation. As to the manner in which a hapten can induce the production of a specific antibody, the experiments of Pauling and Campbell give some indication; these investigators succeeded in transforming in vitro bovine gamma-globulin into antibody by moderate heating, or by adding an amount of alkali to the medium sufficient to start the process of denaturation; they hold that the denaturing causes the globulin chain to uncoil. This gives the hapten—a dye or pneumococcus polysaccharide of type III—a chance to act on the protein, which thus assumes a configuration most stable under these conditions, one complementary to the configuration of the hapten. A subsequent lowering of the temperature of the solution or a restoration of the neutral state effects a renaturation process in the protein, which then represents a specific antibody. On the other hand according to Erickson and Neurath regeneration of denatured antibody protein may take place even without the presence of the specific antigen and they suggest that the difference between normal globulin and antibody globulin may be due to differences in the aminoacid composition of these proteins.

However, important and suggestive as the experiments and conclusions discussed in this chapter are, they do not solve the problem as to the nature of the organismal differentials. After all, it is most probable that the protein molecule as a whole, perhaps with the addition of smaller conjugated groups, represents these differentials, while the larger haptens are characteristic of organ and certain other differentials. Also, in regard to the part of the cell where the organismal differential proteins are situated, no certainty exists. Bensley distinguishes the mobile protein, which is found mainly in the interior of the cell, from plasmosin, a viscous material extracted with 10% NaCl solution and rich in nucleoprotein, and from particulate components of the protoplasm, which are submicroscopic, contain nucleoprotein and phospholipids and are identical with or associated with certain enzymes and viruses. They are in certain respects similar in constitution to the mitochondria, but not the same as the latter. There remain some more solid constituents in the form of membranes and threads. It is very likely that the exoplasm of the cell, which presumably is rich in plasmosin, plays a prominent role in the reactions against strange organismal differentials, and this substance may also contain, or constitute organismal differential proteins; but the possibility cannot be excluded that other cell constituents as well may bear these differentials. The following recent observations of Claude suggest that also the particulate components of the protoplasm may be self-perpetuating and this in turn suggests that they, too, possess individuality differentials.

Claude distinguishes small cell particles (cytoplasmic granules or microsomes) which are suspended in the homogeneous cytoplasmic ground substance and which correspond to the particulate components of Bensley, from mitochondria, Golgibodies and especially from the zymogen or secretory granules. The latter contain more nitrogen and sulphur, but less phosphorus than the microsomes, but both are composed of phospholipids and ribonucleoproteins; there is moreover some indication that the secretory granules contain a material similar to the microsomes and both of these cell constituents may have therefore a common origin. In contrast to these cytoplasmic cell components in the chromosomes, which are the most important constituents of the nuclei, thymonucleic acid is a significant part, but Claude suggests that both these types of nucleoprotein, the cytoplasmic as well as the nuclear, may have the ability to reproduce their constituents by autocatalysis.

The prominence of the nucleoproteins in various components of the cell becomes of special interest if we consider the probability that some viruses, including agents which are involved in the production of tumors, are of a nucleoprotein nature.

Chapter 10

Is It Possible by Experimental Means to Change Organismal Differentials?

INDIVIDUALITY in the sense in which this term is used in our daily life is considered essentially as a fixed condition, and the criteria of individuality most commonly used are certain structural and functional peculiarities of those parts of an organism which are readily perceived through the sense organs. Yet, this mosaic individuality is not as constant as it might appear; to some extent, the parts constituting it may be under environmental control and therefore modifiable. However, we have recognized that there is hidden beneath these criteria something representative of individuality which under ordinary circumstances is constant, namely, the organismal differentials, and the individuality differentials in particular. But we have also, on various occasions, referred to experimental findings which might suggest a certain modifiability of the organismal differentials, and which are, therefore, apparently opposed to the fixity which is characteristic of this type of individuality. Yet an analysis of these data showed that there was no reason for assuming that an actual change in the organismal differentials had taken place; on the other hand modifications in the reactions against organismal differentials were noted in many instances.

This interpretation accords with the genetic origin of the organismal differentials; they are determined by the genes of the fertilized ovum acting in association with the cytoplasm of the growing and of the adult tissues. While the type of the organismal differentials produced by or inherent in the tissues of a certain individual or species is constant, except if germinal mutations should alter the genetic constitution, the amount of these differentials produced might vary under different conditions. Furthermore, the sensitiveness of a tissue against homoio- or heterotoxins and against strange host cells might undergo some modifications under various conditions. Changes in the growth momentum of tissues, or specific adaptations of a tissue to strange substances may occur and changes in the intensity of reactions against strange organismal differentials may be observed under certain conditions. After we have now considered all the principal data concerning the interaction of blood and tissues and their various constituents, those belonging to the same individual, as well as homoiogenous and heterogenous ones, it might be of interest, to consider connectedly the main observations which may have a bearing on the problem of the modifiability of organismal differentials.

1. We have observed that after homoiogenous transplantation of cartilage of guinea pig or rat, the lymphocytic reaction, which may be quite pronounced in the first three weeks following transplantation, instead of becoming

stronger if the transplant is allowed to remain in the host for several months, as a rule actually becomes weaker, and also the reaction of the connective tissue decreases considerably in intensity in the course of time. We may assume that this decrease is due either to changes taking place in the host, which becomes accommodated to the strange individuality differential of the transplant and therefore reacts less strongly to it, or to similar changes in the transplant, which ceases to produce the differential with full strength. These changes would therefore be of an adaptive character, or they might be due to an injury to the transplant, resulting from the long-continued action of a strange environment; however, we would not, under these conditions, have to deal with actual alterations of the organismal differentials, but merely with certain modifications of their manifestations.

2. A much further-reaching change in the nature of the organismal differentials has been assumed by Rhoda Erdmann and Gassul to occur if amphibian skin, in a first period, is cultivated for some time in vitro and then transplanted; they believe that under these conditions it is possible to alter the individuality as well as the species differentials of the transplant and to make it more similar to that of the host and thus to improve the chances of successful transplantation. For this purpose these investigators cultivated skin for a considerable length of time, first in plasma and tissue extract of its own species, then, step by step, they changed the type of plasma and extract, until it approached more nearly in constitution that of the host organism; they believed that by this procedure they had succeeded in increasing the compatibility between transplant and host, and moreover, it was found that the greater the distance in relationship between host and transplant, the longer the time required to effect the transformation of organismal differentials through preliminary growth in vitro; this interval could therefore serve as a measure of the nearness or distance of relationship between host and transplant.

In the first experiment of this kind Rhoda Erdmann cultivated embryonal skin of birds in vitro for from ten to twelve days in homoiogenous plasma, and after transplantation of this tissue into defects in the skin of living homoiogenous adult hosts it was observed that the transplant remained alive longer than corresponding tissue that had not been explanted previously. Moreover, the graft no longer called forth as strong a lymphocytic and fibroblastic reaction as does ordinary homoiotransplanted avian embryonal skin. Rhoda Edmann concluded that the individuality differential of the embryonal skin had been changed through cultivation in vitro. Mammalian tissue, on the other hand, which had also been explanted in a first period, was soon absorbed after homoiotransplantation. Gassul, in continuing these experiments, noted that if skin of an adult frog, which in tissue culture can remain alive for as long as six weeks, is kept for several weeks in vitro in frog plasma, it continues to live after subsequent homoiotransplantation into the skin of another frog for longer than thirty days, and during this period behaves like an autotransplant, no reaction developing around it. Conversely, if a piece of frog skin has been kept for some time in vitro in foreign serum

or plasma and is then homoiotransplanted into a living host, it is cast off after two to five days. Gassul assumed that cultivation in a heterogenous medium altered the tissue in such a way that it assumed a heterogenous character. As far as homoiotransplantation is concerned, he concluded that the preceding explantation in a homoiogenous medium caused an enhancement of the individuality of the transplanted tissue. However, the success of the homoiotransplantation under these conditions might rather be interpreted as signifying that the individuality differential of the transplant, or, rather, the intensity of its production, has been weakened, and it elicited therefore a weaker reaction on the part of the host. Furthermore, Gassul's conclusions are based on a very small number of experiments in which tissues were homoiotransplanted and only some of these successfully. As to the action of heterogenous plasma, especially that of warm-blooded animals, on tissues kept in vitro, this procedure seems to diminish the success of a subsequent homoiotransplantation by causing injury of the tissues.

Subsequently, Rhoda Erdmann undertook heterotransplantation of anuran skin after a preceding cultivation in vitro, in an extensive series of experiments. While, according to the author, normal adult skin of urodeles can be readily transplanted to other urodele species, it is very difficult to accomplish such a result in anuran species. The first of these experiments concerned the transplantation of skin of Rana esculenta to Rana temporaria, and vice versa. Later, pieces of skin from farther distant species were used for grafting, following a previous cultivation in vitro. By means of this procedure an exchange of skin between different families was made to succeed to some extent; thus, skin of Bufo and Bombinator could be transplanted to Rana esculenta, which proved to be the most suitable heterogenous host. Bufo skin was first cultivated in a mixture of Bufo plasma and Bufo spleen extract, then in a combination of Bufo plasma and frog extract, and at last in a mixture of frog plasma and frog extract. Skin thus prepared and afterwards transplanted into adult Rana esculenta was found living and united with the skin of the host even as late as fifty days, and not only the transplanted epidermis but also the skin glands of Bufo survived under these conditions. In the case of the skin of Pelobates, cultivation in vitro for a period of twenty-four days was required before it could be successfully transplanted into Rana esculenta. It is of interest that in some experiments of heterotransplantation into farther distant hosts, hemorrhage killed the host, owing apparently to toxic effects exerted by the transplant.

In interpreting these results we have to consider several possibilities: (a) In vitro, skin undergoes regenerative growth. It is conceivable that transplantation of regenerating skin gives better results than transplantation of ordinary resting skin although in our earlier experiments we did not observe that homoiotransplantation of regenerating skin in the guinea pig differed essentially from homoiotransplantation of normal skin. (b) If skin is cultivated in vitro, it is only the epidermis that is active and grows, while the underlying connective tissue remains inactive and may become detached from the overlying epithelium. Thus after transplantation of this tissue, the

strange epidermis is in more direct contact with the host tissues and is better supplied with blood by the underlying capillaries; this might possibly improve the chances of survival. (c) There is another possibility which has been suggested by Bytinski-Salz. Various kinds of amphibian skin contain glands which secrete poisonous substances. By cultivating the skin in vitro, the poisons may have been extracted to a large extent previous to transplantation, or perhaps a depression in the gland activity and a corresponding diminution in the production of the toxic substances following transplantation may have been brought about. Still, there remains the possibility that cultivation in vitro may have induced a change in metabolism of the skin, which enhanced its transplantability. This change may have been either of a non-specific or of a specific character, dependent upon the kind of plasma in which the skin had previously been cultivated. Similar successful experiments with human skin have more recently been reported by Stone and others; other surgeons, however, did not notice an improvement in the results of homoiotransplantation through a preceding cultivation of the tissue in vitro.

3. More recently, experiments have been made by Lumsden, in which the temporary growth of a mouse tumor in a rat, or of a rat tumor in a mouse, changed the tolerance of tumor tissues in vitro to the corresponding heterogenous sera, in which they were subsequently immersed, in such a way as to suggest that by the growth in the heterogenous species they had apparently lost their own organismal differentials and assumed the characteristics of the foreign species. Thus a mouse tumor, after growing in a rat, had become resistant to serum from a rat which had been immunized against mouse tumor, but at the same time it had become susceptible to the serum of a mouse immunized against rat tissue. However, after transplantation of such tumor cells into rat and mouse, they grew only in the latter; they still behaved therefore as mouse cells and had not really changed their organismal differential. It must then be assumed that changes of a secondary nature in some unknown manner had reversed the reactions towards immune sera. Of a somewhat similar nature are the experiments of Kimura, in which also growth in vitro seemed to induce a change in tissues, but in this instance the change became manifest even in the living organism. However, the experiments of Albert Fischer indicate that no real change in organismal differentials occurs in tissues growing in strange media in tissue culture.

4. The growth energy of tumors undergoes various adaptations in the course of serial transplantations. As a rule, it increases gradually following the first few transplantations, until finally a tumor may grow successfully in hosts in which at first negative results had been obtained, sometimes even in heterogenous hosts. It has been made very probable that the antigenic properties of cells from spontaneous tumors (Dmochowski) and of leukemic cells from spontaneous cases (MacDowell) may change within certain limits in the course of serial transplantations, and that they may thus differ in their reactions, in certain respects, from analogous cells which had not been subjected to such treatment.

If we consider all these experiments together, there is no necessity for

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which apparently depended upon the individuality or species differentials of two organisms.

7. Somewhat comparable changes to those observed in protozoa have been induced experimentally in various kinds of bacteria, and especially in pneumococci. Through serial passages through animals belonging to a susceptible species the virulence of bacteria can be raised. Similar changes have been noticed in the case of viruses. Thus the effects of the virus of poliomyelitis on mice and rats can be greatly increased by serial transfers to mice; but at the same time the virulence has become greater thereby also for guinea pig and Rhesus monkey (Jungeblut and Sanders). In bacteria many kinds of socalled "dissociation" have been observed and produced experimentally; from apparently fixed bacterial forms, bacteria with different characteristics have developed and these new types have remained constant. Modifications of bacteria have also been produced under the influence of bacteriophage or related substances. Increase in virulence for one host species and decrease for another species may follow serial passage of microorganisms or viruses through a certain species of animals. In the case of viruses, this has followed cultivation on the chorio-allantois of chick embryos. However, these modifications in the effects or reactions cannot strictly be attributed to changes in the organismal differentials of bacteria or viruses.

Dawson observed, in 1919, that when bacterium coli was cultivated through many generations in culture media, which differed from the usual ones in their fat and protein content, definite peculiarities developed, which distinguished the strains thus produced from the original ones; especially noticeable was a specific change in the character of the antigens, which after injection in rabbits called forth the production of immune agglutinins; accordingly, the character of the latter was also changed. More specific were the changes which Burnet produced in a strain of B. melitensis. In this case, it was the association with a heat-agglutinable paramelitensis strain which transmitted to the melitensis strain characteristics similar to those of the paramelitensis and modified the antigenic character of the B. melitensis.

But the most striking results have been obtained with pneumococci. Pneumococci were formerly classified into four types, which differed, above all, in the character of the complex carbohydrates contained in their capsules. More recently, Group IV has further been split into twenty-nine additional types. In addition, it is possible to distinguish within at least some of the different types between smooth (S) and rough (R) colonies. The bacteria from smooth colonies possess their typical capsules and behave therefore in a characteristic type-specific way. The pneumococci from rough colonies, on the other hand, have lost their capsules, and with them their type specificity. Furthermore, there exist within the cell-body proper of the pneumococci proteins of a specific character. It can be shown that type specific S pneumococci can be transformed into R pneumococci by cultivating them in homologous type-specific immune sera. According to Griffith, this transformation from the virulent S forms into the avirulent R forms may take place

assuming that, following these various procedures, a transformation took place in the specific structure of the organismal differentials, but there is evidence for the conclusion that the transplantations altered the cells to such an extent that (1) phenotypic changes occurred, perhaps of a cytoplasmic nature, which insured a greater resistance to the injurious effects of certain hosts, or (2) the growth energy of the cells was increased, or (3) the production and the diffusion of the organismal differentials of the grafts were quantitatively diminished, or (4) the transplants were modified in such a way that their reaction towards other individuals or species were altered by means of secondary mechanisms without any actual change taking place in the character of their organismal differentials.

5. If we include in our analysis not only the tissues of higher organisms, but also primitive, unicellular organisms, we find further analogies to the above mentioned phenomena. It is known that in protozoa, changes in the resistance to injurious chemicals as well as to high temperatures can be produced, and that these may be transmitted to successive generations; likewise, apparently spontaneous variations occur in these organisms and extreme types of this kind can be selectively propagated. Especially striking are the experiments in which parasitic protozoa, such as trypanosomes, were made resistant to immune sera, to drugs, in particular, also to certain dyes, acting specifically on these organisms. These adaptive changes can be observed in vivo as well as in vitro. Likewise, in free-living protozoa, for instance in paramaecia, new biotypes can be produced, which may differ structurally as well as physiologically from the original type. Effects of this kind are associated with chemical changes such as the antigenic constitution in trypanosomes, as indicated by the acquired power of the organism to transform a poisonous form of a chemical into a less poisonous one. These effects may be transmitted by heredity to many asexual generations; but they seem to be lost ultimately, especially under conditions of sudden changes in the genetic constitution, such as those taking place at the time of conjugation or endomixis. Whether these persistent modifications (Dauermodifikationen of Jollos) are due to true gene mutations and therefore comparable to real changes in organismal differentials, or whether they are due to cytoplasmic alterations from which a return to the old equilibrium would take place in the course of time is not certain.

There is, therefore, in all these changes the question involved as to their permanence and also as to the respective role of cytoplasm and genes in their causation. However that may be we are directly concerned with such induced modifications or mutations only in so far as they affect the relations of different races and species to one another.

6. In this connection we must also again refer to the experiments of Reynolds, who succeeded in modifying the reactions of various protozoan organisms towards each other by adding to the culture media in which they were kept, fluids from culture media in which other organisms of the same type had previously multiplied. In this way he could change the behavior of pseudopodia belonging to different individuals and thus alter a reaction

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gradually, so that intermediate forms develop, which are able to produce a trace of soluble specific toxin and cause immunity in the mouse. Subsequently, it was found by Avery and Dawson that these R pneumococci may regain their specific properties and again become typical S forms if they are grown in anti-R serum, that is, in serum of a rabbit that has received repeated injections of the heated R organisms and has developed immune bodies against the latter; even normal hog serum may act similarly to anti-R rabbit serum. The S pneumococci which were recovered under these circumstances were always of the same type as those from which the R forms were originally derived. This indicates that the R forms with which one had to deal in these experiments had retained their type specificity, although they had lost their capsules, and with the latter, the type-specific carbohydrates. Thus, a type I pneumococcus which had been converted into the non-virulent R pneumococcus again became a fully developed type I pneumococcus after reversion to the original S form. In this case, therefore, the R forms still possessed, potentially, their type specificity, which under these conditions was presumably localized in the central bacterial body.

However, the experiments of Griffith and Dawson seem to indicate that a still furthergoing change, one of type, is possible. They observed that if a few living R pneumococci are injected subcutaneously into mice, together with very large numbers of killed virulent S pneumococci of a type other than that from which the R forms were originally derived, there may be cultivated in many instances from the injected mice after death, pneumococci of the type to which the killed S organisms belonged, which had been used for injection.

A similar change can be produced even in vitro. Thus when very small particles of R pneumococci cultures were added to suitable culture media containing killed S pneumococci, of a type other than that to which the R cells belonged, S forms developed, which were of the same type as that of the killed S pneumococci. This result is obtained with special readiness if a little anti-R serum is added to the culture media. According to Alloway, the same effect can be noted when instead of adding killed S bacteria, as such, a heated cell-free extract of the S bacteria is used. We have here, apparently, results analogous to those which Reynolds obtained in protozoa. The nature of the substance in the extract, which stimulates the R forms to synthesize the particular polysaccharides involved, has not yet been determined. However, it cannot be the soluble type-specific carbohydrate itself, because the addition of this substance in a purified state does not produce such a transformation.

Of a related character are the experiments of Veblen, who grew microorganisms such as streptococcus viridans and bacillus typhosus for several generations in dilute horse serum and then was able to demonstrate agglutination of these bacteria by an anti-horse precipitating rabbit serum in high dilution, the microorganisms losing at the same time their ability to agglutinate on addition of their own specific agglutinating sera. In this case, a radical change in the organismal differentials of the bacteria, which their agglutination of the latter by horse serum suggests, can be excluded; but we

may have, perhaps, to deal here with a coating of the outer ectoplasmic layer of the bacteria with the serum, which would confer on this layer the characteristics of the foreign serum.

Also, observations of Thomsen, Friedenreich and Hallauer, may have some bearing on this problem. Thomsen (1927) showed that it is possible to change human erythrocytes, irrespective of the group to which they belong, in such a way that they can be agglutinated by human serum, even if the latter does not have a definite relation to the blood group of the red corpuscles used. This change can be affected by exposing the erythrocytes to contact with certain bacterial cultures or their filtrates. Thomsen and Friedenreich explained this effect by assuming that under these experimental conditions a new specific receptor "T" is produced in the erythrocytes. In the normal sera of certain dogs, sheep, hogs, rabbits, guinea pigs, and also of certain mice, there may be found a T agglutinin acting specifically on the experimentally produced agglutinable substance. The T agglutinin is distinct from the normal group-specific agglutinins, anti-A and anti-B. Hallauer, with the aid of immune agglutinins specific for this agglutinable substance, was able to prove that a T receptor had actually been newly formed in red corpuscles of man, as well as of certain other animal species, which had been exposed to such bacterial filtrates. The corresponding immune agglutinins could be absorbed by such erythrocytes, and there is, moreover, some indication that the species differential of these corpuscles is also concerned in the production of these immune bodies, the antigen, presumably representing a combination of a special agglutinable factor and an organismal differential, acting as carrier. As usual, the carrier must be of a heterogenous nature, in respect to the species which is being immunized. In this case, therefore, there would have been experimentally effected a change of a character which, while determined by nuclear genes, affects a cell no longer possessing a nucleus. This result cannot depend upon a somatic mutation of a gene, but it must be due to the alteration of a factor localized in the cytoplasm. Inasmuch as red blood corpuscles do not multiply, the acquired characteristic in this instance is not transmitted to successive generations of cells; however, it is quite conceivable that a cytoplasmic change, acquired by cells which have the power to propagate, might be transmitted to many successive cell generations.

The mechanisms, to which may be attributed these experimental changes produced in single cells and in tumor tissue, are not yet understood, and the findings here reported do not contradict the concept that organismal differentials in higher organisms depend on the genetic constitution of the individual and find expression by means of reactions which presumably take place in cytoplasmic structures. Alterations in these manifestations are therefore not necessarily caused by changes in genetic factors as such, but by modifications which environmental conditions produce in cells and tissues.

On the other hand, it cannot be excluded at present that certain persistent modifications produced in parasitic or also in free-living protozoa may be due to genetic changes caused by adaptative alterations which were induced by environmental conditions. The fact that they are liable to be lost particularly

at the time when the gene sets undergo marked changes, suggest the possibility that gene mutations may be involved in these processes. However, the strictly adaptive natures of such modifications, as well as the fact that after all they are, as a rule, not permanent, makes it more probable that they are not due to genetic changes; they would not therefore represent changes in the organismal differentials.

From all these observations it may be concluded that there is no evidence that in higher organisms an actual change in the constitution of the organismal differentials occurs; but changes may take place in the quantity of differentials which are produced and in the character of the reaction against these differentials. On the other hand, in certain unicellular organisms, in which the criteria used for the definition of organismal differentials in higher organisms cannot be applied, changes have been observed, which in certain respects may perhaps correspond to modifications of the organismal differentials.

Part VII Organismal Differentials, Organ Differentials, and Evolution

THE STUDENTS of evolution, paleontologists, systematists, biochemists, and also geneticists, have used the various tissues and organs, their structure, chemical constitution, their functions, as well as certain peculiarities of the whole individual as subject matter for their investigations. They also studied the mutual structural, functional and chemical adaptation of the organs and tissues within an individaul or species, as well as their adaptation to the milieu in which this individual or species lived. They analyzed, therefore, the history of the mosaic characteristics of organisms in tracing the evolution of species. In a general way, it may be stated that evolution has led to a gradual increase in differentiation and specialization of tissues and organs and to a more and more intricate interaction of the organ and tissue constituents of the organism. On the other hand, the study of transplantation of tissues, together with serological investigations, has led to the concept of organismal differentials, which concerns the differences between individual, species, orders and classes as such, and indicates their relationships. Organismal differentials also have undergone an evolution, which likewise has resulted in their increasing differentiation and specialization. At first only the coarser differentials, those of classes and orders, can be recognized; the mutual compatibility between different organisms and their parts is therefore relatively greater in primitive organisms. Gradually, a refinement took place in these organismal differentials; they became more individualized, until in the end the stage was reached in which the individuality differentials determine and regulate the interaction of the tissues of which the organism is constituted, and in which each organism represents an autogenous system; in this condition an equilibrium between the constituent tissues and organs of an individual exists only if they all possess the same individuality differential, which is autogenous within each individual. Both transplantation of tissues and serology have led to this conclusion. However, as we have pointed out in the preceding chapters, at present it is not possible to deal with the organismal differentials as chemically isolated substances; we merely study the reactions which reveal their presence, and into these reactions variables may enter, which may make it difficult to determine whether certain constellations are due to the lack of certain organismal differentials or to other variable factors which prevent these differentials from becoming manifest. Still, the evidence on hand renders at least very probable the conclusion that the lack of the finer reactions in the case of the primitive organisms is actually due to the lack of the finer organismal differentials, and that it is due largely to this factor that the range of transplantability is wider in the phylogenetically more primitive

classes of animals than in the higher ones. There are indications that an evolution in the organismal differentials has occurred independently of each other in plants as well as in animals.

However, while in higher animals there has developed concomitantly with the refinement of the organismal differentials a very pronounced integration of the various organ systems into one connected, finely balanced mechanism, in plants so marked a degree of integration has not taken place. The individual parts of a higher plant remain much more independent of one another than the parts of a highly differentiated animal. Inasmuch as in the case of animals a certain parallelism exists between the differentiation of organs and tissues and their integration into a whole organism, on the one hand, and the differentiation and specialization of the organismal differentials, on the other, the question may be raised whether, correspondingly, the organismal differentials are less finely developed in plants and whether the latter possess individuality differentials. The readiness with which grafting between two organisms can be carried out in plants seems to indicate that individuality differentials do not play a significant role. If they do exist, then a greater resistance of the grafts to strange organismal differentials or a less strong reaction of the host against the transplant covers up these finer differentials. But, there is reason for assuming that in the course of evolution not only the mechanisms, which make possible the manifestations of the finer differentials, undergo a gradual development, but also that the substances, which serve as differentials, undergo a corresponding evolution. Thus, Steinecke found that antigens obtained from more primitive plants, when injected into rabbits for the production of precipitins, are less differentiated than are those obtained from higher plants. Accordingly, large group reactions predominate in algae; likewise, in cryptogamous plants the differences in the constitution of the proteins between larger groups of plants are, as yet, slight. In phanerogamous plants on the other hand, the specificity in the character of the proteins, as manifested in the precipitin reactions, is greater. It may therefore be concluded that in lower organisms in plants, we have to deal not merely with less finely developed reactions against antigens or organismal differentials in general, but also with less well developed and differentiated organismal differentials and antigens. We may assume that the same conclusion applies to animals and that here, also, there is a parallelism not only between phylogenetic evolution and the fineness of reactions against organisms, but also between phylogenetic evolution and the development of organismal differentials and the corresponding antigens.

As to the chemical substratum in which the changes take place, which parallel the structural and functional evolution, there is justification for believing that proteins, either as such or in combination with other groups, play the most prominent role; and it may furthermore be held that the phylogenetic evolution of the organismal differentials was associated with an increasing complexity of protein substances. However, our knowledge as to such evolutionary changes in the proteins is as yet very slight. Kossel has shown that in fishes the nuclei from which the sperm chromosomes are produced consist of combinations of

protamines or histone-like substances with nucleic acid, and that the protamines and histones of the sperm differ in different species of fishes as regards the nature and grouping of their amino-acids. But there is apparently no direct parallelism between the chemical relationship of these substances in different species and the phylogenetic relationship of the latter, and two different species of Salmonidae may contain identical protamines. Furthermore, according to A. E. Taylor, Gay and Robertson, and Wells, it is not possible to produce species-specific immune bodies against protamines and histones. But, these simple proteins develop perhaps from more complex nuclear proteins which may be present in the cells from which the spermatozoa are derived, or else these may be admixed to the suspension of spermatozoic substances which are antigenic and bear organismal differentials. Accordingly, through injection of fish sperm into rabbits, Kodama could obtain specific immune sera, which reacted with the spermatozoa of their own as well as of related species, but not with the extract of fish muscle, and which were therefore organ- or tissue-specific; yet these immune sera possessed also organismal differentials as shown by the fact that they reacted in a graded way with the spermatozoa of different species of fishes, in accordance with the phylogenetic relationship of these species. We must then assume that substances other than protamines act as antigens in this case. In the sperm of mammals we find instead of the simple protamines or histone-nucleic acid combinations in fishes, more complex nucleo-proteins. These substances may serve as antigens, which call forth immune reactions against the organ as well as against the organismal differentials or their precursors contained in the sperm. Also, other animal proteins may have a species-specific character.

Other instances are known in which differences in species are associated with differences in the structure of proteins, although these data do not contribute to an understanding of the evolutionary changes which have taken place in the proteins. Thus, the constitution of globin in the hemoglobin molecule differs in different species in regard to the relative amounts of amino-acid nitrogen present and the proportion between lysin and histidin, on the one hand, and arginin, on the other hand. Osborne and Gortner observed a certain parallelism between the chemical relationship of the seed proteins in the wheat ard barley groups and the phylogenetic relationship of the species in which they occurred, and these differences in chemical relationship correspond to immunological reactions (Wells).

As to the chemical constitution of individuality differentials, it is almost certain that here, too, proteins are involved. The individuality differentials cannot as a rule be detected by chemical or immunological analysis of blood sera, but under certain conditions they have been detected in erythrocytes by means of immunological methods. The great difficulty in the chemical analysis of the individuality differentials lies in the fact that the preparation of proteins of cells and tissues for study in many cases causes their denaturation and this change injures the individuality differentials, which evidently possess very delicate chemical characteristics.

Not only does the phylogenetic evolution tend in the direction from coarser

to finer differentials and towards individualization, but a parallel evolution takes place also during the ontogenetic development. Here, also, the organs and tissues evolve from a relatively simple substratum and this process is accomplished through the interaction of chromosomes and their genes with preformed cytoplasmic structures with the aid of evocators and organizers.

Likewise during embryonal development, the organismal differentials undergo a transition from less individualized precursors to more individualized differentials. The processes which lead to this ontogenetic differentiation exhibit certain remarkable similarities to those noted in the phylogenetic evolution, or expressed differently, the embryonal development of phylogenetically farther advaneed organisms resembles and repeats the embryonal development of phylogenetically earlier stages, as though the number of mechanisms which living matter can use in attaining the advanced stages of development is limited and determined by the actual stages through which the phylogenetic development has passed.

In the same way in which organs and tissues and their differentials, as well as organismal differentials, develop during embryonal processes, so also during regeneration of some of the more primitive organisms certain aspects of the phylogenetic evolution may be repeated, although to a still more restricted degree than during ontogeny. Regenerating tissues of adult urodele amphibians behave in some respects like embryonal tissues. Parallel to the increase in the extent of organ and tissue differentiation, with advancing regeneration there is a decrease in transplantability. The earlier, less differentiated embryonal and regenerating tissues are still more plastic and adaptable to environmental factors than the farther advaneed stages, in which the organ and tissue differentials are more fixed and in which there is a greater tendency on the part of organs and tissues to develop by means of self-differentiation. In higher organisms the ability to produce new organs during regeneration is lost. There is again, therefore, noticeable here a relation between the differentiation and fixity of organs and tissue differentials and the increasing refinement of organismal differentials, both of these processes leading to a greater immutability and fixity of the organism as a whole.

However, notwithstanding these similarities there is one very significant difference between phylogenetic and ontogenetic evolution. The former starts with a primitive substratum, in which the specialized organs and tissues and the finer organismal differentials of the higher organisms are not yet preformed. Chromosomes and genes, as well as cytoplasm of the primitive organisms, differ greatly from those of the higher ones and this is true of the germ cells as well as of the cells of the adult tissues. On the contrary, in the fertilized ovum of a higher organism, all the chromosomes and genes and the precursors of organ (tissue) differentials and of organismal differentials are present, and these precursors of organismal differentials differ from those of other individuals and species. The organs and tissues, and also the organismal differentials, merely mature in the course of ontogenetic development, whereas they are newly created in the course of phylogenetic evolution.

Various types of specificities in chemical and morphological structure and

in the function of organisms and their constituent parts may be distinguished. There is the specificity of the organismal differentials, and in particular of the individuality differentials, on which depends the autogenous equilibrium of a higher organism; the latter determines the controlled interaction between adjoining cells and tissues and makes possible the integrity of the organism, guarding it against invasion by strange organisms or their parts. The mutual adaptation of tissues and also the specific adaptation between the bodyfluids and the cells and tissues of an individual depend upon this specificity of the individuality differentials. Upon such a specificity depend also primarily the reactions to strange organismal differentials, which serve as antigens and cause the production of the various kinds of antibodies as a means of defense against the intrusion of foreign elements into the individual organization. This specificity of the organismal, and especially of the individuality differentials, is the basis of the "essential individuality."

There is, secondly, the specificity of organs and tissues that interact within the individual and this specificity depends upon the differences in the chemical and structural constitution of the parts of which the organism is composed. Various organs with interlocking functions form primary organ system, in which the correlation between the functions of individual organs may be controlled by nervous mechanisms or hormones, or both. These primary organ systems are then combined into larger systems, until in the end the whole organism acts as a unit. The interaction of the various organs within the same individual is so perfect that it seems to express the underlying "wisdom of the body," as Cannon has so aptly called it. The totality of these organs and organ systems, together with other structural and functional peculiarities of the organism, represent the "mosaic individuality."

The organ specificities and various structural and functional characteristics of an individual or species have developed in the course of evolution and they exhibit a gradation corresponding in a general way to the phylogenetic relationships of individuals and species. It is possible to reconstruct, to a certain extent, phylogenetic systems by means of these organ and other structural characteristics. Certain constituents of organs or tissues may therefore exhibit, in this respect, characteristics similar to those shown in the typical manner by the organismal differentials, from which they differ, however, in their chemical structure and in the fact that they are restricted to a single organ or part of the body and are not inherent in all the constituent parts of an organism, as are the typical organismal differentials. They may be designated as secondary or accessory organismal differentials. It seems that various organ differential substances may detach themselves from the stem of the organismal differentials at different stages during phylogenetic as well as during ontogenetic development, and that these substances may undergo an evolution more or less corresponding to the systematic relationships; but still this differentiation in other respects may develop independently of phylogenetic relationships. Indications of such a process may be noted in certain food reserves, for instance those of the yolk of the egg and of the seeds of plants; here there is a development of substances which more or less corresponds with the

phylogenetic relationship of the species, but which, as in the egg yolk, does not take a course quite parallel to the evolution for instance of the serum proteins. In many cases it is not possible to distinguish between these accessory and the primary organismal differentials, because of the impossibility of carrying out the necessary experimental tests. Also, certain products of organs, such as enzymes and hormones, so far as the latter are proteins, may possess organismal differentials; but whether these differentials are of the first or second type is unknown.

There are present in various species other systems of differential substances in which a much more limited parallelism exists between the chemical nature of these substances and the phylogenetic relationship of the species. This is the case, for instance, in some groups of higher organisms in which the primary blood-group differentials bear specific relations to certain constituents of the blood sera. Blood groups of the same kind are found in man and in certain anthropoid apes, but these close similarities are lacking if man and less nearly related species are compared. A still more limited parallelism is shown between phylogenetic relationship and the distribution of the Forssman heterophile differentials. Such partial parallelisms may be observed also between the evolution of organ differentials and of the interactions between certain organs on the one hand, and phylogenetic relationship of the species on the other. We have referred already to the observation of Sherwin, that phenylacetic acid is detoxified in more primitive organisms, including monkeys, by conjugation with glycine, leading to the formation of phenaceturic acid. In human beings, it combines with glutamine and is eliminated in the urine as phenylacetyl glutamine; and according to Power a chimpanzee behaved like man. Another example of a parallelism between the nature of metabolic processes and phylogenetic relationship is the following: creatinine phosphoric acid plays an important role in muscular contraction, but it is almost exclusively found in vertebrate muscle; in invertebrate muscle its place is taken by arginine phosphoric acid. However, there are two important exceptions to this rule. Creatinine phosphoric acid is also found in the muscles of some echinoderms and of Balanoglossus. The latter is believed to represent a form transitional between invertebrates and vertebrates.

The distribution of urea and uric acid conforms only partly to phylogenetic relationship; but there is a definite connection between the production of urea or uric acid in certain classes or species of animals and the distribution of the enzymes arginase, xanthine oxidase, urease, allantoinase and allantoicase. Such a partial relationship applies also as far as the distribution of hemoglobin is concerned. It occurs in the erythrocytes of all the vertebrates and in the plasma of annelids and molluscs. In the corpuscles of annelids there occurs the pigment hemerythrin, and in the plasma of gastropods and cephalopod molluscs, as well as in the plasma of crustaceans and other arthropods, there occurs hemocyanin. From such systems all kinds of transitions may be found to an entirely random distribution of substances, without regard to phylogenetic relationship, as for instance, that found in the case of the heterophile

antigens, with the exceptions already mentioned, as well as in the case of the melanin pigments.

There are, then, morphological and metabolic characteristics of tissues and organs which, to a high degree, seem to be correlated with the gradation and relationship of the organismal differentials; but these characteristics are limited to certain organs and tissues and they are not common to all the tissues, organs and organ functions of an organism. There are other structural and metabolic characteristics of tissues and organs which are only partly correlated with the organismal differentials and with the phylogenetic development, and still others are only slightly or not at all correlated. But, it is only in the larger groups, such as classes, orders, genera, that the morphological and biochemical evolution of certain organ and tissue systems can be correlated with the course of the phylogenetic evolution and with the evolution of organismal differentials. If we study individual organisms, the distribution of organ and tissue characteristics is independent of the individuality differentials. In brothers and sisters there are structural, biochemical differences in certain organs and tissues, as well as psychical differences, which do not parallel the relations of their individuality differentials; this is true also of the distribution of the original blood groups. There is reason for the conclusion that the organismal differentials have a closer and much more direct correspondence to phylogenetic relàtionship than the organ and tissue differentials.

There are, in addition, certain specific functional or structural relationships between some cells and tissues, which very closely correspond to the relationships between the organisms from which these cells and tissues are derived, but which are not identical with the primary, typical organismal differentials. Thus in some instances there exist between germ cells, spermatozoa and ova, or between germ cells and certain somatic tissues, specific relations which make possible the distinction between autogenous and homoiogenous relationship. Likewise among infusoria there are mechanisms which enable these organisms to distinguish the autogenous, homoiogenous or heterogenous nature of parts of these organisms. We have in this, as well as in other similar cases, to deal with processes which have developed not in the direct line of phylogenetic evolution but in side branches and which are peculiar to them; in particular, in unicellular organisms, it is not certain what role is played by genetic factors and what by cytoplasmic modifications in such mechanisms. In all probability many other mechanisms of a similar nature exist, which make the interactions between different organisms or between parts of them specific for species, varieties or individuals. In different cases the mode of manifestation of these specificities may vary, and likewise the mechanism by means of which the mutual adaption of cells and tissues is produced may vary.

An organism consists, then, ultimately of systems of graded substances, some of which possess a very great organismal-specificity while others are almost exclusively organ-specific, and still others show combinations of organismal and organ differentials, varying quantitatively in different instances. In addition, there occur substances which are specific for a certain species or

individual, but show no direct connection with organismal differentials, nor can they be strictly considered as organ differentials, an instance being the four original blood groups. These substance-specificities are paralleled by structural specificities. The full, complex interaction of such specificities including the marked organismal-specificity in the relationships between the various parts of an organism and between different organisms represents perhaps the most characteristic feature of organisms: these specificities all increase with increasing evolution. To such specificities of substances and structures there corresponds a specificity of the reactions which take place between the different constituent parts of an organism, and between different organisms or parts of them. These specific reactions include the normal correlations and functional reactions between different tissues and organs within the individual organism; they include furthermore, the reactions of immunity and anaphylaxis. On such specific reactions depend also, ultimately, certain functional correlations between the organism and his environment, by means of which the environment is distinguished from the individual's own organism and, within certain limits, is reshaped by the latter, and some constituents of the environment are transformed into organismal and organ-specific constituents of the organism. On these specific reactions are contingent, as well, those functions which make possible the transmisson of specificities to new generations. The problem of evolution consists largely in the analysis of the mode of development of these specific systems, on which the specific reactions depend.

It is due to the combined effects of the individuality differentials and the various systems of organ differentials and to the resulting organ functions, that the fullest development of individuality in the highest organisms takes place. But the individuality differentials, and the organismal differentials in general, as well as the chemical and morphological structure of organs and their functions, are themselves determined primarily by genetic factors. As to the nature of these genetic factors, these differ in the case of individuality and organ differentials. The various characteristics of an organ, as a rule, are determined each by one or by a restricted number of genetic factors which are transmitted in accordance with the laws of Mendelian heredity, although various complications may arise in this process. This predominating effect of a single gene or of a few genes, or of certain changes in chromosomes on the ontogenetic development and on the functions of organs and tissues holds good, although during the various stages of embryonal life and also during adult life the cells of the most diverse tissues and organs contain, as far as it is known at present, complete and identical gene sets. It must be due to the interaction of the gene sets with a variety of cytoplasmic structures that the differentiation of tissues and organs within the same organism can take place. On the other hand, there is reason for assuming that the individuality differentials depend upon a very large number of genes or, perhaps, on the entire gene sets. This conclusion rests on several observations, but especially on the fact that while, with progressive close inbreeding by means of consecutive brother-and-sister matings, the similarity of these differentials in

two individuals belonging to the same inbred family or strain can be gradually increased, it is very difficult to achieve complete identity as it exists between different parts of the same organism. This identity of the individuality differentials of different tissues and organs in the same organism can be demonstrated, notwithstanding the existence of great differences between different tissues and organs.

It is primarily the difference in individuality differentials of the individuals belonging to the same species which causes the reactions of the host against the transplant, the local as well as the distant reactions, and which also may cause immune reactions in an animal after introduction of tissue or its constituent substances or of bodyfluid belonging to a not closely related individual; parts of the same individual do not elicit either a contact or a distance reaction after transplantation; nor do autogenous substances elicit an immune reaction, except perhaps parts of the body which, in certain respects, are separated from and strange to the other parts of the organism, and in particular products of degeneration, which may differ in constitution from the living parts. Organ differentials and artificial partial antigens, as a rule, function as full antigens only in combination with strange individuality or preferably with strange species and order differentials. It is especially the strange organismal differentials which interfere with the integrated function of the host organism into which they are introduced and which make it possible for the host to react also against specific structures other than organismal differentials.

As to the progressive evolution in structure, chemical constitution and function of tissues and organs, and in the constitution of the whole organism, it is assumed by geneticists, and also by some other students of evolution, that this is caused by mutations, alterations in chromosomes and genes, in association with processes of segregation and selection. If the conclusion is accepted that mutations are the primary means through which organisms change in the course of evolution, then it would be further necessary to assume that changes in organs, caused by mutations, will affect also the organismal differentials in the course of time. As far as the individuality differentials are concerned, there is reason for believing that these depend, as already stated, upon very many genes and it may therefore be assumed that a change in a single gene, which might be sufficient to induce a modification in the structure and function of a certain organ or tissue, would not alter the individuality differential noticeably, or only to a very slight degree; but repeated mutations might produce a more marked effect on the individuality differential. Such modifications in the genetic constitution would in many instances affect only superficial mechanisms, which do not control vital processes in the adult, and they would affect, first, the late stages in embryonal development. Secondarily, however, such changes might influence also other mechanisms in the organism and thus alter a variety of characters. As to mutations which result in slightly further-going changes, such as those which have led to the transformation of gray Norway rats to "Mutant Albino" or to "Curly Coat," which were observed by H. D. King, even these do not seem to change the organismal dif-

ferentials to any great extent, as is indicated by the exchange of tissues between these races. But it may be held that certain gradations exist in the relations between the organismal differentials and these organ mutations, which are superimposed upon numerous genetic differences which already exist between different individuals, families and strains. However, in addition the possibility would have to be considered that changes in organismal differentials depend on specific mutations, which do not affect one single organ or tissue but certain characteristics common to all organs and tissues.

The analysis of organismal differentials from the viewpoint of evolution is bound up with the analysis of the genetic differences between races (strains), subspecies, species and genera, and of the mode of origin of these genetic differences and of speciation. As a rule, multiple genetic and chromosomal differences distinguish races, subspecies, species and genera. These differences are caused by an accumulation of mutations, which consist either in changes taking place in chromosomes or in genes. They occur in a population which is spread out over a certain geographic area. Subsequent processes of selection, which vary in character in different environments, seem to lead to the formation of geographic races and species and may explain the adaptation which exists between these groups and the environment in which they live; at least quite commonly certain environmental characteristics are associated with certain structural and functional characteristics of the organisms inhabiting certain areas (F. B. Sumner), and these associations between environment and constitution of organisms seem to develop independently in various places and in different races or species belonging to the same wider unit. Such differences between races, species and genera are greatly aided by lack of interbreeding between adjoining populations and these obstacles to the interbreeding may be produced by a variety of factors. In case they are due to structural and functional differences in sex organs, such differences are of no greater importance in the distinction between species than differences in other organs and organ systems; but the consequences of differences in sex organs leading to sterility between adjoining populations are much more important as far as speciation is concerned. There remains still the problem as to whether the adaptations noted between environmental conditions and structural and functional peculiarities of organisms are caused by random mutations followed by selective processes, or whether, in unknown ways, certain ecologic conditions exert a certain influence on the character of mutations which take place in these environments.

While the number and nature of structural differences, and in particular, also the interferences with interbreeding between races (strains), species and genera may be taken as indicators of the degree of difference between the organismal differentials of these groups, the real relationship between these organismal differentials can be determined only by the direct tests for organismal differentials and the structure of the latter is the real criterion of the nearness or distance in relationship between individuals and species. As to the character and number of genes which differentiate various species and whole groups of species, we may refer to the investigations of Landsteiner and

Miller, who compared the occurrence of the ordinary human agglutinogens and of M and N agglutinogens in man, the anthropoid apes, as well as in Old World monkeys and New World monkeys. They could trace in this way the development of certain genes corresponding to these agglutinogens within a limited range of the evolutionary process. Man and anthropoid apes are most closely related; accordingly, they have agglutinogens A or B, or both A and B, in common. The distribution of A and B differs in different species, but it also differs in different human individuals. Factors A and B have not been found among Old and New World monkeys; but among certain New World monkey species and among Lemuridae a factor may occur, which is related to but not identical with human B. M and N agglutinogens occur as alleles in man, but in chimpanzee a combination (MN) has been found, which has not been observed in man. Again there occurs in Gibbons and New World monkeys a factor similar to but not identical with the human M. It has been assumed that progenitors for A and B are present in common ancestors of these species and that B is perhaps the older factor.

Irwin tested by means of a series of immune sera, the number of genes which were common to various species of pigeons. In comparing two of these species he concluded that each of these has a number of genes which the other species do not possess, and in addition there is a set of genes which both species have in common. Accordingly among the Old World species of pigeons there are sets of genes which are characteristic of each species, and other genes are shared by these species. The same applies to the New World species of pigeons, and a third set of genes is shared by Old and New World pigeons. These conclusions would imply that the gene constitution of each class, family, genera, species, strain, and still more, of each individual, is extremely complex, consisting of numerous sets of genes which two different groups share and of others which distinguish them. The nearer these species or groups of species are to one another, the greater is the number of genes they have in common, and the further distant they are, the smaller is the number of genes which are identical. These conclusions are based on the assumption that each agglutinogen is associated with or determined by a particular gene; however, there is the possibility that each agglutinogen is determined by more than one gene. Furthermore, it is only one type of phenotypic characters and one type of genes which have been considered in these investigations, namely, those which determine the agglutination of erythrocytes by specific immune sera. But there are innumerable other characters which are independent of the agglutinogens of erythrocytes and if the gene-determiners of these characters were also included, the complexity in the genic constitution of tissues would become still much greater.

It seems plausible that mutations causing very fargoing changes in organs and acting on earlier embryonal processes may modify some of the more basic organismal differentials, which developed first in the course of evolution, while mutations affecting the constitution of organs, which were more recently acquired and which are of a less fundamental nature, may modify the individuality differentials. In general, it seems that the more similar two

organisms are to each other in their structure and chemical constitution, the more similar as a rule are also their individuality differentials, and that to more fundamental differences in chemical constitution and structure correspond furthergoing genetic differences in the organismal differentials. In the course of phylogenetic evolution the finer organismal differentials developed gradually; at least they became manifest only in the course of advancing evolution. Likewise, as evolution progressed, an increasing differentiation and specialization of tissues and organs took place. Comparable processes occur during embryonal development; but here the organismal differentials develop from precursor substances, the complexity of which increases parallel to the increasing complexity of the organs and tissues and organismal differentials in the higher organisms.

However, during ontogenetic development endstages are reached in which again a decline sets in in the manifestation of the more specific organismal differentials. The more the cellular substance proper of organs and tissues diminishes and the more the paraplastic and intercellular substances predominate, the more specific is the organ and tissue differentiation and the less prominent become the finer organismal differentials, the species and individuality differentials. The character of the lens of the eye, and presumably also that of keratin and of other specialized structures which no longer possess the typical cellular constitution of the tissues from which they originated exemplify this change. But this is found only if certain serological reactions are used as tests for the presence of organismal and organ differentials; by means of contact and distant effects of transplanted tissues it is still possible to demonstrate the presence of individuality differentials in such tissues, at least in the case of the eye lens. It is therefore probable that in these paraplastic tissues the individuality differentials have not been entirely lost, but that their existence cannot be demonstrated by the less sensitive serological methods; this may be due to the fact that they have relatively diminished in quantity perhaps on account of the increasing preponderance of the organ differentials.

Evolution is essentially the history of the adaptations between organisms and their environment and between constituent parts within the organism, the non-adapted organisms being eliminated. But there has been an evolution not only in the development of the organisms, their tissues, organs, and their organismal differentials; there has been an evolution also in those processes which lead to the decline of these organisms, such as ageing, tendency to disease, and death, all of which are manifestations of the lack of perfect adaptation. Primitive organisms possess great plasticity in organ formation and they possess the ability to restitute the whole organism under the influence of internal and external environmental factors. This plasticity is associated with a lack of the manifestation of finer organismal differentials and therefore with a lack of individuality. The higher organisms constitute much more rigid, fixed wholes, which exhibit very fine individuality differentials. In the higher organisms the organ systems have become more complex in structure and function, and in their interaction with other organ systems. The primitive

organisms, because of their great plasticity and ability to produce organs and to restitute whole organisms, are potentially immortal, in the restricted sense which applies to beings living on a planet and in a universe over which they have no control. The higher organisms, because of their rigid organization and lack of plasticity, because of their greater individualization, have lost the power to restitute the whole organism and to be potentially immortal; at best, only small constituent parts still possess such power, and this can be realized only under artificial experimental conditions. Higher organisms are more readily discoordinated and disorganized. The delicate mechanisms of adjustment to one another which their organs and tissues have developed, no longer enable them to repair more extensive injuries experienced under the influence of inner and outer environmental factors, to undergo compensatory regulations and to propagate asexually. They have acquired senescence and associated diseases in the attainment of individuality and one of the prices they paid for individuality was the potentiality to immortal life.

But while there is a parallelism between the ascending evolution of organismal differentials, the specialization of organs and tissues, the increasing rigidity of the organism, and the apparent inevitableness of senescence and death, it is, in the first place, the increasing complexity in the structure, constitution, and metabolic and functional interaction of tissues and organs rather than the increasing specialization of the organismal differentials which is responsible for these pathological consequences of ascending evolution. As a result of the greater differentiation of the organs and their increasingly intricate interaction the organs became more delicate and, in the course of time, they were no longer quite adequate to the performance of their functions, and this change becomes more and more cumulative with the advancing years of the individual. The relative proportion of reversible cyclic and irreversible non-cyclic processes is more and more altered to the advantage of the non-cyclic with increasing age of the individual.

Many processes in nature are cyclic, but other processes, as all those subject to the second law of thermodynamics, are nonreversible, proceeding only in one direction. The disintegration of radio-active substances is nonreversible, although under altered conditions also a creation of the latter may occur. In organisms the essential functions must be cyclic; this is the case with circulatory, respiratory, alimentary functions, with sleep and hibernation, with the proliferation of certain tissues. The sexual processes are also at least partly cyclic, but they sustain the life of the species rather than the life of the individual. However, these cyclic processes are grafted on an irreversible process of a non-cyclic character, on one continuous process, starting with birth and leading to growth, maturity, old age and death. This process, irreversible as far as the individual is concerned, is the basis of cyclicity in the species. But, also, the species may be subject to non-cyclic changes and will be destroyed in the end, when external conditions cause at an early age a decline in the organisms and make propagation impossible.

We may then regard disease and death as manifestations of insufficient adaptation between the different constituents of an organism and between or-

ganism and environment. The process of gene mutation itself, which presumably plays so important a role in evolution, is frequently the source of maladjustments and therefore many mutations are lethal; in other instances they may lead to malformations and abnormalities in metabolism and function, or they provide the basis on which certain environmental conditions can act in an injurious way. Pathological mechanisms, or mechanisms arising in response to injurious conditions and tending to counteract them, have their phylogenetic history as well as normal mechanisms. We should therefore be able to trace the origin and evolution of disease processes phylogenetically in the same way as the evolution of normal structures and functions; but only the beginnings have been made in this direction.

Thus, we have attempted to trace the development of thrombosis, a partial or complete occlusion of blood vessels, which in mammals depends upon changes in the vessels, in the character of the blood flow, and in the composition of the blood. From the primitive process of agglutination of amoebocytes in Limulus and the subsequent combination of this with processes of coagulation of blood in higher invertebrates, this condition ascends through the lower vertebrates to its full complexity in mammals. But thrombosis, which represents a disease and has destructive consequences for so many higher organisms, is closely associated with processes which, instead of being injurious, have an adaptive value, such as the prevention of bleeding following injury. Furthermore, it is of interest in this connection that the mechanism leading to the primitive thrombus formation as found in Limulus has much in common with that underlying tissue formation, as we pointed out in a preceding chapter. In a different field of pathology, Metchnikoff has shown that it is possible to trace phylogenetically the activity of phagocytes, which play so important a role in inflammation and in immunity, from simple processes of digestion in primitive organisms to the most complex reactions against injurious material in mammals.

In a provisional way we may distinguish four types of inadequacies or diseases which are however not sharply separated from one another but overlap to a certain extent. 1) The ultimate inadequacy, which becomes manifest in the course of life in the differentiated and the rigid organisms belonging to the higher classes of animals, is also the cause of the imperfect utilization of certain important food factors, as is also the lack of tolerance by certain organs for food factors which are necessary for other kinds of tissues or organs; thus, disharmonies in the organism set in. 2) Likewise, inadequacies in the relations between the individual and his natural or social environment may lead to such disharmonies and these, too, have had their evolution. 3) A third type of disharmony causes a disease which may also be traced phylogenetically; it consists in changes in certain tissues, which make them assume a cancerous growth and thus invade and destroy the organism in which they originated. Cancerous growth has, so far, been observed only in the relatively rigid organ and tissue systems of the vertebrates; in those organisms, in which restitutive growth processes lead to the formation of organs, to multiplication of the individual animals, or to colony formation, excessive growth stimulation should not cause

the development of cancer. The latter represents, then, apparently a disturbance of the organ and tissue equilibrium, which has ensued from the increasing differentiation and specialization of tissues and organs and from the increasing rigidity in the constitution of the whole organism, which took place in the course of evolution.

4) A fourth type of disease is due to the struggle between complex higher organisms and various types of parasites, especially bacteria, protozoa and various viruses. In this case the reaction of the host against the invader is due in part to the effect of specific toxic substances, which injure certain tissues and organs of the host; also, the direct destructive effect of parts of the host by parasites may play a role in this disease process. But there are viruses which, instead of causing a primary destruction, may induce cancerous growth processes in certain hosts and in certain tissues of these hosts. Moreover, parasites possess organismal differentials which differ greatly from those of the host, and these differences may disequilibrate the latter and thus lead to disease. However, the organism which, as the result of inadequacies in its own constitution and in its interaction with the living and non-living environment, receives injuries and becomes diseased, is not merely a passive agent; it also responds actively to the injurious factors, and these reactions may be the cause of new diseases superimposed upon the primary ones. Local reactions of the host, in the form of so-called inflammatory processes, may cause a sclerosis (cirrhosis) of certain organs, with serious consequences for the economy of the organism as a whole. But also thrombosis, and even cancer in certain respects, may be considered as reactions of the organism against abnormal conditions; furthermore, immune processes directed against strange substances are, in many cases, beneficial, causing the death of the invading parasite, or helping to destroy strange organismal differentials or to convert the latter into the differentials of the host. However, in other cases they may be destructive for the host. This occurs if reactions of a similar nature to the immune processes lead to states of anaphylaxis or various kinds of allergy. In these conditions, organismal differentials may also play a part and there is reason for assuming that the sensitiveness to strange organismal differentials becomes greater with furthergoing differentiation and specialization of the organismal differentials; likewise, the destruction of organs becomes increasingly serious with the increasing differentiation of organs and their increasing inability to restitute the lost parts with advancing evolution. Thus, with progressing evolution disease processes may preponderate over restitutive processes, although both go hand in hand.

The relations between the host and the various organisms which live on or in the host may be that of symbiosis or parasitism. As to the role which organismal and organ differentials play in these relationships, in some instances host and symbiont or parasite may belong to the same species, and this occurs in plants as well as in animals; but as a rule they belong to very distant classes and usually the host is phylogenetically a much higher organism than the parasite or symbiont. Also, the degree of specificity in these relationships varies greatly in different cases. There may be an adaptation

of parasite or symbiont to one particular kind of host, or an adaptation to a number of phylogenetically related hosts or to very diverse hosts, or the two latter adaptations may exist at the same time. Parasites of animal or plant origin as well as viruses may live and propagate in or on very distant organisms; there does not need to exist an exact relationship between the organismal differentials of host and parasite corresponding to phylogenetic evolution.

However, under certain conditions organismal differentials may play a certain role in determining the invasion of the hosts by the strange organisms. The relationship between the organismal differentials of host and parasite or symbiont may resemble that of certain organ and tissue differentials, or the distribution of blood-group or Forssman differentials in various species. But whatever the significance of organismal differentials in these relationships may be, parasites and symbionts are usually adapted to definite host species and often also to definite organs or tissues within a certain species. There exists therefore a marked specificity in the relations between host and invader. This specificity may be so great that it is possible to distinguish between different strains of hosts by determining the kind of parasites or symbionts which live on or in them, and conversely, to distinguish between nearly related parasites or symbionts by determining the host on which they are found. The mutual relationship between the organismal differentials of host and parasite may be one of the factors which determine the interaction between these two organisms; this interaction does not depend however on the organismal differentials of either host or parasite alone. In this respect the relationship between host and parasite resembles that between host and transplant, which depends on the organismal differentials of both host and graft.

There are indications that the specific adaptation between host and parasite or symbiont may be due partly, at least in some cases, to the presence of certain substances in these two organisms which are specifically adapted to each other. Furthermore, related parasites may contain related antigens, which may call forth the production of antibodies showing cross-reactions with the antigens of these parasites. A very instructive observation pointing to the presence of specifically adapted substances in host and parasite, which make possible this condition of parasitism, has been made by Welsh, who found that various species of mites which live between the gills of Anodonta and other mussels are positively heliotropic when they are removed from their normal habitat. Addition of extract of the gills or of fluid from the mantle cavity of the species on which they live makes this heliotropic reaction negative, and it is only extract or fluid from the species to which they are adapted which has this effect and not substances obtained from other species of mussels.

As mentioned, there is noticeable in many cases also a distinct organ- or tissue-specific adaptation between host and parasite or symbiont. However, in this respect also, great differences exist in different parasites; some are adapted to a single organ or tissue, others can live and multiply in several or in

the large majority of the tissues. This organ-specificity suggests the possibility that a definite species distribution of the parasites may not be due to the specificity of the organismal differentials of the hosts, as for instance in certain cases in which the parasites live and propagate only in a single species or in a very few species, but is due rather to peculiarities which organs in different species possess. The term organismal differentials would therefore be used here in a wider sense.

While there exists in the relations between host and transplant frequently an organismal- as well as an organ-specificity, in some instances the organ-specificity, in others the organismal-specificity may predominate. But these specificities are not always rigidly fixed; they may be modifiable through serial passages of the parasite in a host species other than the one to which it has been originally adapted. Gradually a change may take place in the relative virulence of the parasite for various host species; this change in species-specificity can be obtained also by means of many passages through the chick chorio-allantoic membrane or the chick embryo, and not only the species-specificity may be diminished or altered by this procedure, but also the organ-specificity may be markedly decreased. These effects can be studied very well in various viruses. There is therefore noticeable, here, a great similarity between the behavior of certain viruses and of tumor transplants; the latter can become adapted to new hosts through many consecutive passages in different hosts. Moreover, in heterogenous tumor transplants, a good growth has been observed in the chorio-allantoic membrane of the chick; the same is true of the growth of heterogenous normal tissues. In both viruses and tumors an adaptation to a new host occurs in the course of long-continued transplantations and the chick embryo and chorio-allantoic membrane seem to lack the power to injure viruses or tissues and tumors possessing heterogenous organismal differentials. It remains still to be determined how far these similarities in the behavior of microorganisms and viruses, on the one hand, and mammalian as well as avian tissues and tumors, on the other, depend on similar mechanisms.

The evolution of the organ systems and that of the organismal differentials has led to the formation of very complex, rigidly integrated organisms, in which the various organs and tissues are highly specialized. These processes have also resulted in an increased differentiation between organisms belonging to the same species and therefore in an increased individualization. In this individualization, different organ systems have had an unequal part. The generative system is less important for the individual than for the continuation of the species, and the so-called vegetative organ systems are essential for the life and function of the individual, but are not individualized to the highest degree. It is the nervous system in its interaction with the other organ systems, and especially with the endocrine organs, whose development in the course of evolution has made possible the greatest individualization. Increasing differentiations in the nervous system, in its cooperation with the hormone system, have made possible the coordination and correlation of the functions of the various organs and tissues belonging to the same organ system and

the functions of various organ systems with one another; in this sense the nervous and endocrine systems have made possible the integrated, very complex organisms which have gradually developed in the course of evolution. But in addition, the nervous system has developed in still another direction; it has become the organ system which, above all others, controls our relations with the environment. The meaning of the environment, its variety and its richness, depends for each species and for each individual to a large extent on the constitution and function of the nervous system. While the vegetative organ systems make possible the life and functioning of the organism in a rather simple physico-chemical environment, the nervous system has become the organ which by way of the sense organs, transmits to us a picture of the environment and which represents the environment within us. Furthermore, the development of the nervous system in the course of evolution has made possible the creation of the most individualized type of environment, the psychical environment, consisting in memories and, in the end, in the production of the environment in thoughts and thought-emotion complexes. This psychical environment has increased in richness and significance with advancing phylogenetic evolution, but it has reached a high development only in man. In accordance with the advance in differentiation and individualization of the human organism, his relations to the environment have also become more differentiated and individualized and thus many new points of contact have been created between him and his non-living as well as living environment. These contacts have affected the natural struggle for health and life with the non-living environment and with other less highly differentiated organisms; they have affected also the social competitive struggle with other human beings for material and psychical goods. In the natural struggle, evolution has led to the building-up of the physical-chemical sciences, of technique and industry; and in the social struggle, notwithstanding many retrogressive movements, there has been, on the whole, a development in the direction towards a greater freedom and understanding in the spheres of political, economical and social relations and towards an increasing valuation of the dignity of the individual, as well as a beginning development of the psychical-social sciences.

While man has thus lost the potentiality to immortal life, he has obtained a greater and richer individuality; he has also gained a life of abstract thought that may help to shape or, if he so desires, to replace the real life and the universe, and he has won a certain degree of consistency and continuity in existence through the persistence of thought and through its transmission to successive generations.

Evolution has laid the basis for and has actually led to changes in the significance and working of the natural struggle and natural selection. A certain point has been reached in evolution where it has become possible to replace the crude and brutal struggle, which at least partly controls and dominates the fate of the more primitive organisms, by a civilization which in the end tends to become universal; thus development has taken place by way of intermediate cultural stages, in which particularistic interests and

aims functioned instead of the later, more universal ones. Ultimately there tends to be created a humanistic mode of life, which can develop only at a level of evolution reached by man. At this level, the physical as well as the psychical factors of life attain a balance in which the wellbeing, bodily and mental, of the individual will best be guarded.

Thus the contradiction between our concept of our personality and what has been considered as the ultimate master of the fate of species and individuals, namely, the natural and social struggle, will be diminished as far as the latter are in conflict with the physiological needs and desires of the individual, and only such safeguards will be established in this process as will make possible the avoidance of retrogression and degeneration, bodily as well as mental, in human society, without abandoning the principles and ideals of civilization and their practical application to civilized life. In man, the thought-life predominates and the realization of ideas may give the deepest meaning to his existence; if the ideas represent true abstractions and generalizations, if they are in harmony with science, they are no longer concerned solely with narrow circles of individuals, but with all humanity, and finally they may comprise the universe; they may then become the possession of mankind. Thus the conflict between the wishes of the individual and his fate in the natural and social struggle will, in the end, be mitigated and the struggle for the survival of the fittest will be replaced by the knowledge and understanding of a civilized society, in which a conscious direction of further evolution may take place.

In the course of evolution there have then developed organisms in which individuality and its constancy depend upon three factors: 1) the structure, function and interrelations of organs and tissues; 2) the function in particular of the nervous system especially that on which memory is based and which gives distinctiveness and continuity to the highest organism, man; and 3) the action of organismal and individuality differentials.

As to the first factor, organs and tissues are in a constant flux from early embryonal life through early extrauterine, to adult life and old age; there is a greater difference between the structure and function of an embryonal tissue and organ and the corresponding tissue and organ in the same individual during old age than between the organ or tissue characteristics of two different individuals at comparable ages; it is only the potentiality of organs and tissues to undergo a certain development in the same individual which is characteristic of the individual and constant. Regarding the significance of memory in the maintenance of individuality in the psychical sense, the effect of the latter is imperfect and limited in time; it cannot fully function as the expression of individuality. There remains the third factor, the action of organismal and individuality differentials, which is completely characteristic of the individual and which maintains its identity in the same organism; this factor then represents the essential individuality, whereas the first two factors merely support this essential individuality; each of these factors has passed through a definite evolution which we have correlated with the evolution of the other factors.

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Part VIII The Psychical-Social Individuality

Chapter I

The Physiological Basis of the Psychical-Social Individuality

IT IS PREEMINENTLY the phylogenetic development of a certain organ complex, the nervous system, that made possible the phylogenetic development of individuality in the psychical-social sense. An individuality similar to that of the higher mammals, and in particular of man, does not yet exist in the more primitive organisms. In some of the earliest forms of animal life there may be found instead of single individuals, groups of individuals, colonies, which later may separate into single organisms. Gradually within single organisms a differentiation of the component parts occurs and these differentiated parts become more and more integrated and coordinated in such a way that a complex whole results. The process of coordination takes place largely through the nervous system in conjunction with the hormones, but it seems that the nervous system itself may exert at least many of its functions by means of specific hormone-like substances, which it transmits along the nerve paths. As a rule, however, the hormones are carried to distant areas by way of the lymph and blood channels and help to influence and correlate the various parts of the organism. In this process of coordination there are involved reflexes in which nerve fibers, ganglia cells and hormones, which circulate in the bodyfluids, may cooperate. In a wider sense, we may include among the hormones also certain contact substances, which are given off by one tissue and which act on an adjoining tissue.

Corresponding to the increasing integration of the individual, the nervous system becomes more complex. This system is lacking in protozoa and in sponges; however, in certain ciliate protozoans fibers exist, to which the function of conduction of stimuli and coordination of movements have been attributed. Sponges may be cut into many small particles and when these are joined together at random they form a whole new organism. The definite beginnings of a nervous apparatus consist in a system of nerves without a central set of ganglia. The primitive nervous system of the simplest invertebrates is not yet polarized. The simplest type of a central nervous system is found in the echinoderms; in the starfish, for instance, there is a circumoral nerve ring consisting of nerve fibers with attached scattered ganglia cells, but as yet without real ganglia. This nerve ring functions as an organ which co-

from the sensory stimuli received from the environment depends, therefore, not only on the character of the sense organs, but also on the central nervous system.

In correspondence with this evolution of the nervous system there seems to take place also an increasing complexity in the production and action of hormones. The number of hormones which have so far been demonstrated in invertebrates is relatively small and their presence has been noted principally in the higher types, particularly in the arthropods. To mention some of the hormones and their actions which have been studied best: The experiments of Koller and Perkins, and others, have shown the existence of a hormone which is involved in the movement of pigment in the chromatophores of crustaceans; it is produced in the eyestalk in the sinus gland. Analogous hormones may affect also the chromatophores of various classes of vertebrates (G. H. Parker, F. B. Sumner). Kopeć and others found that in Lepidoptera the supraesophageal ganglion controls pupation. In addition in Hemiptera and Orthoptera molting and pupation are induced by hormones originating in nervous ganglia. According to Wigglesworth a hormone given off by the corpus allatum inhibits metamorphosis. In Drosophila a hormone responsible for pupation is given off by the larval ring gland which is situated between the two hemispheres of the larval brain (Hadorn), and Bodenstein has moreover made it probable that this gland induces also the differentiation of organs into the imaginal state. The last mentioned effect may however be an indirect one and as in the case of other hormones already discussed, the changes induced by the ringgland do not depend solely on the nature of the hormone, but on a balance between hormone and the state of the recipient tissue. Various other hormones probably exist in invertebrates; there are in particular indications in crustaceans and also in other invertebrates that hormones may affect the development of secondary sex characteristics; furthermore the socalled gene hormones might be mentioned in this connection. As in the case of vertebrates so also in invertebrates no strict species specificity of hormones seems to exist and there are even indications that invertebrate hormones may perhaps affect vertebrate organs and conversely certain vertebrate hormones may affect invertebrate tissues.

The relative scarcity of the hormones so far discovered in invertebrates as well as their distribution in different classes suggests that an evolution comparable to the evolution in the central nervous system may have taken place also in the case of hormones; this would be in accordance with the simpler structure, the less developed differentiation, integration and coordination, the more limited organ functions in these more rudimentary animals as compared with the conditions found in vertebrates. However, there are no exact data available which would make possible at the present time a definite comparison of the number of hormones present in a tissue unit in vertebrates and invertebrates, and it will be a task for future research to trace the phylogenetic evolution of hormonal regulations.

In addition to these central mechanisms there are the peripheral receptors, the sense organs, which in response to physico-chemical factors emanating

ordinates the movements of the various rays of the starfish (Romanes, A. R. Moore). Next there arises a segmented nervous system, in which, in addition, there forms a central system of ganglia, and in which each segment of the body contains collections of ganglia cells joined by nerve fibers running lengthwise through successive segments. This is a step towards increasing integration. The segmented plan, on which the nervous system is built, persists even in the highest organisms. At first the developing central ganglia are of relatively little importance, because the different parts of which the organism is composed are still largely independent of one another.

Under favorable conditions certain cells and tissues retain the power to live independently, even in the highest organisms, but the ability of parts of the organism to lead their own existence and to restitute the whole organism when separated from the rest, diminishes step by step; likewise, the power to regenerate organs or other portions of the animal lessens or may become entirely lost. Concurrently with this development and with the increasing complexity of tissues and organs and their greater tendency to coordination, the central nervous system gains in importance and becomes more intricate. However, it is not until the latter has reached a certain stage, after a differentiation of the cortex has taken place in the forebrain, that thoughts may form on the basis of sense impressions and that the ability to abstract and synthesize becomes possible, and that there thus develops the psychical-social individuality in its most complete form. The cortex arises first in reptiles.

The phylogenetic development of the social-psychical individuality is thus paralleled by the evolution of the nervous system with its increasing complications, such as an increase in differentiation and segregation of parts of nerve cells and fibers in localized areas, the formation of certain projection systems connecting the peripheral sensory receptor organs with subcortical ganglia and the connections of the latter with the peripheral effector organs and later with the cerebral cortex. It is also paralleled by the development of systems of association fibers within the cortex of the brain, the formation of distinct ganglia within the more or less diffuse neuropil, and by the stratification and individualization of the cortex. At the same time there seems to remain everywhere a less well defined neuropil, consisting of shorter neurons and collateral nerves; it infiltrates the other, more localized parts diffusely and to this tissue has been attributed, by Herrick, the integrative activities of a lower type, as for instance, the maintenance of the tonus in more primitive organisms, as well as those of a higher type. According to this view, these latter processes would not essentially be localized, as are certain sensory projection nerve fiber systems, the motor nerve fiber systems with which they connect, and, although less obviously, the association fiber systems. Hand in hand thus with the differentiation and individualization of certain separate mechanisms, new connecting, centralizing systems develop. In accordance with the increasing complexity of the organization in general, the number of the primary simple reflexes increases, the complexity of their interaction likewise increases, and they then extend and become converted into complex, associated, conditioned reflex systems. The effect eventuating

But in vertebrates distinctions between individuals may be recognized. It is especially the development of conditioned reflexes which leads to the differentiation between individuals of the same species, because individual distinctions of a finer type depend upon individual experiences of a special kind, and conditioned reflexes seem to play only a very small part in primitive organisms under natural conditions, where we have essentially to deal with "forced movements" based on non-conditioned reflexes.

Even in the apparently most highly developed invertebrates, the social insects, bees for example, rigid reflexes and complex reflex systems, instincts, determine the very complex modes of their behavior. While environmental changes may have a slightly modifying influence, in general these animals are guided in a reflex manner by scents, colors, and certain spatial characteristics; also time factors, such as the position of the sun, may perhaps affect their activities. While this interaction of some environmental factors with a complex organization may lead to very complicated modes of behavior, still, the latter remain essentially rigid and forced and are largely non-modifiable. However, memories seem to play a certain part in the behavior of these organisms, and further complications, in addition to those caused by conditioned reflexes, are introduced by the changes taking place at some periods in the life of the individuals, which may lead to changes in the reaction modes. Although to a very limited extent the reactions of these animals have become modifiable, in the main they are rigid and fixed. It is the complexity of these reactions and their social nature, which are the distinctive features in the behavior of the social insects and which raise their behavior to an apparently very high level, making it comparable in certain respects to the social life of higher vertebrates.

While, as we have seen, there are no indications of marked individualization in the strict meaning of this term, sharply defined group differences exist in accordance with structural and functional differentiations within certain species of insects. The various species of ants differ and show gradations in regard to their psychical-social behavior in a way analogous to the differences and gradations in the structure and function of various organs, and in the structure of the body as a whole. In general, the behavior patterns, which are essentially based on inherited instincts, are similar in nearly related insects and differ in further distant species; but in some cases nearly related species may present very different types of behavior, and relatively distant species may show similar types of behavior; for example, certain bees are non-social in their behavior pattern, while insects as distant as bees and ants may have in common very complex social reaction systems.

Fishes, representing the most primitive class of vertebrates, recognize to a limited degree individuals and species. There are species differences in behavior; on the whole, behavior patterns are similar in related, and quite different in more distant species. Individuals belonging to the same species school together, joining their own species in preference to a strange one; and conversely, a certain school receives members of its own species and repels members of a strange species. In the social dominance system, in which there are individuals

from the environment, whether living or non-living, determine the action of animals. As a rule, these sense organs also undergo an increasing differentiation with phylogenetic evolution, although some of them may be developed to a higher degree in more primitive organisms than in man. In general, the mechanistic basis of primitive animal behavior is clearly discernible, but with further phylogenetic advancement and with increasing structural and functional complexities new and more complex processes arise, which may render difficult the analysis of the behavior of the organism as a whole.

The mechanistic character of the behavior of animals was recognized by Jacques Loeb, and it was with particular clearness discernible in the tropistic reactions of animals as highly developed as certain insects. Especially suggestive was the observation of this investigator that slight physico-chemical changes in the environment were able to cause a reversion in the direction of the tropistic reaction.

Simple non-conditioned reflexes represent the functional elements which seem to underlie animal reactions. However, it appears that ganglia of the central nervous system give off, also automatically, stimuli which are transmitted to the peripheral nerves and to the recipient end-organs. This would mean that a specific stimulation by afferent nerves of the reflex arc or by hormones is not required for the function of these ganglia, but that they may discharge stimuli under the influence of non-specific substances or mechanisms which reach them. Yet, it is probable that the important functions of the central nervous system, which determine the behavior of higher organisms, are essentially of a reflex nature. The most important complication which next arose in animal behavior consists in the conditioned reflexes discovered by Pavlov. Even at a very early phylogenetic stage, former actions of the environment may modify the subsequent behavior by means of conditioned reflexes. Thus, learning is made possible. But the importance of these processes is, on the whole, limited in invertebrates, although they seem to be widely distributed. Thus, conditioned reflexes have been shown to exist in polyclad flatworms. It has been maintained that they can be demonstrated also in protozoa; however, A. R. Moore has pointed out that in the latter one may have to deal with a condition analogous to the hysteresis of metals and colloids, in which a longer lasting after-effect of certain treatments can be shown to exist, comparable to certain fatigue phenomena rather than to true conditioned reflexes. The latter exist, however, in larvae of lower vertebrates, as, for instance, in Amblystoma (A. R. Moore). Even in vertebrates they bear a definite relation to the inherited structural and reflex constitution of the various animals and, as stated, represent an addition to these latter. The more varied the simple reflex activities of the organisms have become, the more varied may be the conditioned reflexes which are added to them.

Therefore, behavior at first is rigid and fixed, corresponding to the originally relatively simple structure of the organisms and especially to their nervous system and sense organs. There are no indications that individual differences are very significant in the behavior in the more primitive animals.

female fish does not react against a former mate, but reacts against a new mate, this may merely be due to the loss of a stimulus, owing to former experiences.

The mechanisms underlying the very complex, strictly determined migrations of eel and salmon over very long distances at definite periods of life, are unknown, but there is no indication that higher psychical processes are involved other than those functioning also under other conditions of life. On the whole, the reactions of fishes under given circumstances can be predicted by the student of fish behavior, except in instances in which conditioned reflexes have formed and processes of learning have taken place; in such cases it would be necessary to know certain phases of the history of a fish in order to make the predictability complete. There is no need to assume the existence of free will in the psychical life of fishes. It seems that the greater part of their behavior is determined by rigid simple reflexes and reflex systems, but joined to these are modifiable types of behavior based perhaps on memories of sense impressions and of isolated events, and these may be associated with simple feelings.

The reactions of birds represent also essentially fixed reflex systems, and, on the whole, they are very similar to those observed in fishes, although the signs used by birds are more complicated, insofar as in addition to various visual stimuli, such as movements, postures, colors, designs, and to olfactory stimuli, finely differentiated auditory stimuli enter into their psychical life. Sounds given off by individuals begin already to play a role among amphibia, but they become much more varied among birds; there are specific sounds given off by mates and also by parents and children. Among the birds, too, are found species distinctions, sex distinctions, and well developed sex symbolisms used in the sex life of males and females. There is also a seeking for and claiming of territory in which to live and to breed, and the individual which is the first to claim a given territory has the advantage over those that enter later; the former tends to be the dominant individual. There occur group reactions as well as individual distinctions, and a definite order exists regulating dominance in a group. Furthermore, there are migrations to great distances by birds as well as by fishes, and the flight reflex plays a role in the life of both, as well as in that of animals.

Again, both fixed inherited mechanisms and a certain degree of modifiability of behavior are involved in determining the tendency to complex seasonal migrations, which many species of birds exhibit. In some species this process is due merely to definite organ functions and is independent of experience. However, in other birds only some component parts of the reaction system, which leads to seasonal migrations, depend upon fixed reflex systems, while other parts of it appear to be learned from older birds. In the latter case we have to deal with mechanisms which induce a bird to follow other birds in their movements; in this way conditioned reflexes are set up and the animals learn how to move; thus a mechanism of tradition may develop. Furthermore, we may find individual differences in the intensity of the tendency to migration, the impulse to migrate being much stronger in some

with attributes of graded superiority or inferiority, these gradations serve to distinguish individuals. Females learn to recognize their former mates; they attack strange males of the same species, but do not react against their old mates. Parent fish learn to recognize their young. There are very distinct and fixed behavior patterns relating to the sexual life, preceding the mating act; also, the spawning and brooding functions are rigid, inherited species characteristics. Movements which have the effect of suggestions and lead to imitations of movements play a part in these actions. In the selection of territories and nest sites, color distinctions are involved; but here, also, previous experiences of having lived in an environment with certain colors may influence the choice of the nesting place.

The signs which serve as symbols determining recognition, schooling, sexual acceptance or attack in the social life of fishes, are partly visual, such as recognition of movements and fine distinctions in color designs; but also olfactory stimuli, extracts of skin, wounds, substances extracted from dead fish of the same species call forth avoidance reactions in Phoxinus. The essential behavior patterns are genetically determined species characters; but learning leads to certain modifications in the behavior patterns. If a certain individual is differentiated from other individuals belonging to the same species, this does not necessarily mean that the individual recognition in fishes has the same significance as in man; although the same term is used in both instances, we have probably to deal with processes of a different nature. In fishes the recognition of an individual signifies the sorting out of a fish as representing a child, a former mate, or a certain degree in the dominance series, one fish being differentiated from others probably by means of a single sign, such as a color pattern of the head, a particular movement, or perhaps a certain olfactory stimulus given off by this fish. In man, on the other hand, an individual represents a composite of many different bodily signs and psychical expressions, which have acted on another individual by certain movements, have given rise to certain experiences and have aroused hopes or fears. However, the most primitive and the highest vertebrates—fishes and man—give evidence, in common, that the distinction of individuals and species depends directly on characteristics of organs and their functions, on movements and expressions, and not on the organismal differentials. These organs differ in structure and function in different species and individuals, and the behavior patterns differ accordingly; but both the structure and functions of organs and behavior patterns are connected and correlated with the organismal differentials.

In addition to the reaction modes which we have mentioned, there are some further indications of the ability of the fish to modify the rigid behavior patterns as the result of experience. Frustration or perhaps painful sensations result in an avoidance of certain motor activities, which would have been carried out under normal favorable conditions. But there is no suggestion that reproductions of sensations or events are the essential factors in these learning processes or are the necessary cause of modifications in the way of reaction, although such reproductions may perhaps also participate. If a

the female before copulation can take place. Here, the parents peck only the foreign young, while they recognize and respond to the calls of their own; but in certain other species parents may take care of strange young as well as of their own. The aggressiveness differs greatly among different species of birds and the aggressive behavior may be a complex called forth by definite stimuli. Thus the aggressive behavior of the falcon is not due to a general tendency to attack other birds; but these reactions take place only when a specific stimulus acts on a falcon and releases a chain of reflexes. Perhaps the movement of another bird sets in motion this mechanism, which seems to represent an inherited, fixed character.

There is among birds individual recognition in the same sense as among fishes; yet, in both it is restricted in significance. Individuals are recognized as mates, as inferiors or superiors in the order of dominance, as parent or young, as members of a flock, but not as individuals in the strict sense of the term. Again, it is a single and relatively simple character, such as color, design of certain feathers, or sounds, or perhaps combinations of a few of such signs, which determines recognition. The two partners composing a pair of immature herons may recognize each other after a separation lasting as long as twenty days; voice and feathering serve as signs in this process. Artificial feathering of the head interferes with recognition. Refeathering the head and neck of both members of a pair of young herons does not prevent the recognition of a partner after the lapse of a few hours; if, hower, the refeathered birds have been separated for six or more days, they no longer recognize their partners (Noble, Warm, and Schmitt). Recognition of individuals, as well as of members of the same group and species, depends therefore in birds, as in fishes, on mosaic characteristics, and the processes involved, as such, are fixed by inheritance, as is also the range in which they are modifiable by experience.

In mammals essentially the same reflex systems and instincts are active, which determine the behavior in fishes and birds; these make possible the distinction between their own and strange species, groups and individuals; they play a role in the intake of food, in the sexual life, in the relations between parents and offspring, in the superiority-inferiority relations within groups, and in the fight and flight reactions. The additions to the behavior reactions of fishes and birds which are noted in mammals consist in a greater modifiability of such reactions. Furthermore, the range of sense organs, through which the environment acts, is enlarged; by way of olfactory, visual and auditory stimuli the changes which affect mammals become, on the whole, more varied and often much more delicate than those perceived by the more primitive invertebrates. Memories of things and events help to influence the behavior of mammals, especially of those belonging to the more differentiated species, to a much higher degree than is observed in lower classes of animals and the actions of mammals may be purposeful, in the sense that situations are sought which experience has shown to promise satisfaction of certain instinctive needs. Among these desired activities is the act of playing, a modified reproduction of instinctive activities; but playing is indulged in

individuals within a certain species than in others. Age and sex functions may also modify the strength of the reaction, which, in certain species, is apparently much more developed in younger than in older birds. It seems that in migratory birds environmental changes, arising from seasonal conditions, set in motion a reaction system which expresses itself in restlessness, but the mechanisms underlying these migratory tendencies are as yet only imperfectly known.

The instincts, as well as the kind and range of modifiability of behavior, are inherited characteristics, and, on the whole, they are very similar in closely related species and more dissimilar the more distant the species are; these reactions resemble, in this respect, the organ systems and outer configurations, for instance, in various species of birds which, on the whole, are similar in nearly related species and differ in more distant species. However, there may be differences here too between nearly related species; as, for instance, in behavior connected with courtship and parental care of the young. There exists among birds, also, a well developed group life, the groups consisting of individuals belonging to the same species, and members of one's own group are distinguished from those belonging to strange groups. The stimuli which hold certain groups together may be genetically determined in one species and acquired by experience in another species. Voice, posture and movements may regulate the activities in a certain group. There are indications that even conditions comparable to what may be called suggestion in the human species play a part in the group life, inasmuch as movements of one individual are quickly transmitted to other members of the flock and elicit in them a similar behavior. The movements and attitudes of a male may initiate corresponding actions in the female; in this case sense organs transmit the mode of reaction and induce imitative behavior in another individual. Similar effects of suggestions have been noted also in fishes.

Also in other respects the species characteristics of behavior are to a large extent genetically fixed, although there is a certain range of modifiability in accordance with experience. Fixed species reactions are, for instance, those of cowbirds, which return to their own species even if they have been reared by foster parents belonging to different species. The flocking together of birds of the same species, another fixed mechanism, depends on the inherited functions of organs and only in an indirect manner on the identity of the organismal differentials of the individual birds, although in different species of birds the degree of specificity in the tendency to gregariousness seems to vary. The European and African stork, for instance, flocks each with its own type and the two types do not mingle with each other; but in certain other species such a strict segregation does not take place, members of different species undertaking common flights.

The superiority-inferiority relations may begin very early in the life of birds among nestlings. These reactions depend upon inherited reflex systems, but they may be influenced by experience gained in testing other individuals. As stated above, a stranger in a certain territory tends to be inferior in authority to the first-comer. In herons the male must have established superiority over

instincts which are active in these animals. There is no indication that analytic thought processes occur even in anthropoid apes, and higher types of creative work are apparently outside the range of their mental capabilities. On the whole, the actions of mammals are fixed even from a quantitative point of view. Thus it seems that the distance which must separate a circus trainer and a wild animal in order to avoid reactions of flight in the latter is quite definite. A student of animal behavior, who knows the history of an individual animal, should therefore be able to a large extent to predict its attitudes and behavior in a certain constellation.

Proceeding now from the other higher mammals to man, very pronounced complications in the modes of reactions are observed. Not only does the environment, which acts on us through our sense organs, induce changes which have a much more varied and also more lasting effect on our behavior than in other mammals, but abstraction and synthesis, in which the elements in the environment are separated and then re-arranged in new combinations, become very prominent. Thoughts develop, in which the constituents of the environment may appear in combinations different from those in which they occur under natural conditions; through shifting of these constituents new concepts are formed.

In man we have to deal largely with secondary mental mechanisms, conditioned thought reflexes, which are much more complex than the simple reflexes. Pictures and thoughts enter into these reflex chains which ultimately end in tensions, in motor activity or in inhibitions. Just as a sound, light, color or odor, so a thought, a sentence, or other symbols for more complex experiences in general, can elicit conditioned reflexes. A further complication arises when a thought calls forth other thoughts, thus leading to an extension of thought reflexes. As we remember experiences, so, too, we remember thoughts. Moreover, abstractions and syntheses may have their first origin in sense impressions, but the material with which they deal may have its origin also in our own thoughts or in the thoughts of others; once we have made the latter our own, no distinction exists between the effects of the thoughts of others and those of our own thoughts. Thus thoughts become the objects of abstraction and synthesis. These various trains of thought make connection with the simpler non-conditioned, as well as with the conditioned reflexes, which latter are established much more readily and in much greater variety in man than in other mammals, and the resulting various combinations form very finely balanced systems. Our attitudes and actions are determined by these systems of conditioned and unconditioned reflexes in combination with thoughts, representing primarily true or false sense impressions and, based on these, reproductions of a real or imaginary reality. Or thoughts may function as suggestions, and then they are transferred into actions and attitudes and the content of the thoughts is converted into vivid pictures of reality which rigidly fix actions and attitudes.

Thoughts are especially effective in their function as suggestions if transmitted to us in the form of a direct or implied command; but in some respects, in every thought there is included such a command. On the other hand,

without the existence of situations which would give it a functional significance in the natural or social struggle. This type of behavior is sought for its own sake, for the satisfaction which the instinctive action provides, dissociated from the results to which it would lead if used in the struggle of life. Also, the modifiable modes of behavior, those based on memories or representing conditioned reflexes, are built on the foundation of simple reflexes and instincts; they are an elaboration of these processes. As a result of this extension in the range of behavior, the environment, as far as space, time and the relations to other organisms are concerned, has become larger; the differentiation between individuals has become finer.

However, the reflex and instinctive basis of behavior remains and the response to sense impressions on this primary basis takes place with less delay than when there is an interference by the restraining effects of thought; however, memories of frustrations and pain may inhibit reflex and instinctive actions also in less differentiated mammals. Complex processes, such as the building of a nest for the young, which in more primitive animals are purely instinctive, not directed by modifiable thinking, are largely reflex actions also in mammals. Many years ago the writer followed with interest the nest-making activities of mother rats, which seemingly indicated the presence of intelligence and thought. When this nest-making instinct is active, the mother may be seen running around in the cage carrying every little article that can be used for nest-making to the place where the nest is to be. But if the observer than transfers the rat from the netwire cage to the outside of the cage and allows her to run around it, the instinct to gather material for the nest continues to be active and she now pulls things away from the nest as soon as she reaches it from the outside of the cage, just as readily as she formerly had carried things towards the nest. Returned to the cage, she now again carries back to the nest the things she had taken away from it. No reasoning can be detected in these actions.

While thus, on the whole, the life of higher mammals is still rigid and fixed, nevertheless the plasticity of individual and social activities has become greater than it was in the more primitive organisms. Similarly, the activities of dogs are not essentially creative, in the sense that the constituents of a composite experience would first be taken apart and then synthesized in a new fashion. For instance, a dog looking for a ball which has been thrown into a basket, attempts, in accordance with inherited reflexes, to recover it by scratching the basket with his feet and pushing it along with his nose, without succeeding in obtaining the ball by these means; he does not discover the simple expedient of turning the basket over, and if the basket is turned over by accident, the dog does not readily make use of this experience. A higher stage has been reached in anthropoid apes. The chimpanzee is able to invent new modes of action, to compare new combinations by shifting of mental elements, and thus to accomplish a certain end by means which are not directly of an instinctive character. In this manner the unpredictability of behavior, or what appears as freedom of action, is increased; this increase however, is very limited, and is closely related to the unconditioned reflexes and

conscious thinking and feeling, centering around the "I," which activate in us the thought and feeling that we possess a distinct individuality. But frequent repetition of an experience or of certain reactions, and the consequent habit formation result in a loss of the intensity of thinking, of the ready and extensive association of the momentary thought with other thoughts, and in particular with the "I" concept, as well as of the accompanying emotions, and thus our mental processes change from the conscious to the unconscious state. Any impediment, however, arising in our habitual actions, making them more difficult of performance, again tend to restitute conscious processes. There is a further factor which intensifies our feeling that we are distinct individualities in the psychical-social sense; this is the idea that we have free will, that our actions are in the last instance determined by ourselves, without inner mechanisms or outer environmental factors rigidly controlling our choice. The feeling of freedom of the will is conditioned by the great complexity of the factors and their intricate connections acting on us and directing our reactions.

We find, phylogenetically, a progressively increasing complexity in the activities of organisms and increasing differences between members of the same species, an increasing individualization which reaches its highest development in man. Conditioned reflexes are acquired more readily and in a greater variety, the more highly developed the species; but even in apes the highest degree of apparent freedom of action depends upon a very limited range of adaptation between the aims sought and the means used to accomplish them. Moreover, the reactions in organisms concern the satisfaction of relatively simple needs, both needs and reactions being constituent parts of instincts. These processes remain, to a great extent, mentally dissociated, while consciousness depends upon the ready association of a thought with a large series of other thoughts and with pictures related to it in time, space and content. We forget, therefore, much more readily what we do as a simple conditioned thought reflex than what we do consciously; in the latter case we are better oriented, but also more subject to inhibition.

While in man thought reflexes also have their root in the needs of the organism, the variety of his conditioned reflexes increases greatly in all directions with the increasing number of constellations and suggestions acting on him. This increasing complexity is made possible through the action of the cortex of the brain, which mediates between the environment and the more individualized reactions of the organism. There develop, thus, a multitude of thought-emotion mechanisms and a play of interacting thoughts based on memory, abstraction and synthesis, the result being that our behavior appears unpredictable to others; and it is often inexplicable to ourselves, in that we commonly err in our judgment or deceive ourselves as to the origin of our actions, the causal connection between environmental factors and thoughts, emotions and actions being difficult to establish. The greater the complexity of the factors acting on and in the individual, the greater the probability that non-recurring patterns will occur. These original, unique, unforeseen and apparently unpredictable configurations in the life of the

abstraction and synthesis, resulting from our dealings with the environment as well as with thought processes, may determine our actions and attitudes without regard or even in opposition to their suggestive effect. In this case our thoughts act as true or imaginary representatives of reality. On the basis of our experiences gained in dealing with the outer environmental world or with our thoughts, we make furthergoing abstractions concerning the character of abstractions and synthesis in general and their relation to the environment. Thus logic is built up. The purely logical, rational use of thoughts as determiners of our actions freed from the elements of suggestion and detached from their function as instruments in the natural and social struggle, represents the highest type of human activity and the closest adaptation to reality. But when thoughts are not concerned with the purely intellectual reproduction and interpretation of elements of the universe on a rational, logical basis, they deal with and are instruments in the natural struggle and in the social struggle, or in a combination of both. In this case our thoughts function largely as suggestions rather than as representations of reality and the resulting actions tend the more to be accompanied by strong emotions, the more they are parts of the social or natural struggle. The tendency to emotional response decreases in inverse ratio to the increasing importance of thoughts functioning as symbols of reality.

As a result partly of rational thought, but largely also because of the friction, antagonism and pain developing in the soccial and natural struggle, the concept of the "I," as contrasted with the concept of others and of the surrounding world, develops. The "I" is the individuality in the psychical-social sense. This concept has a very intricate structure, consisting of combinations of thoughts and emotions, memories, hopes and fears. Like all thoughts, it has a complex origin, its sources being within us as well as in the surrounding world. Hence our "I," our psychical individuality, does not admit of a sharp separation between us and others, between ourselves and our environment, although originally the concept developed in contact with and in antagonism to the environment.

Related to the "I" concept is the state of consciousness in our actions or attitudes. Conscious psychical processes are those which form easily remembered combinations with such other pictures, thoughts, emotions and experiences as are close to them in time or space, or have certain elements in common with them. The term "consciousness" is used also in another sense, in order to express the distinction between psychical and bodily processes; our images, thoughts, feelings and emotions are separated by us as conscious processes from the chemical-physical processes underlying them and associated with them. In general, most abstract thinking tends to be conscious because it depends upon large combinations of experiences and remembered analogies; it requires mental exertion, in contradistinction to the relative absence of mental effort connected with thoughts when they function as suggestions or otherwise exist in a relatively dissociated form. When a suggestion or command, direct or implied, enters into our mental processes, it tends to become conscious only if it functions as a disturbing element. It is largely this

greater in birds than in fishes, although the principal reaction types are the same in both these classes, and may be recognized even in mammals. Thus certain birds which were hatched or were reared by foster mothers belonging to a different species, may flock with the strange species; they may even mate with members of the latter provided the association has occurred at a sufficiently early period of life. In this case, as in the induction of mutations, definite sensitive periods do exist in developing organisms, which greatly antedate the time when the reaction takes place. In this way, through learning, even the species instinct may to some extent be overcome.

A further indication of the complexity of behavior in certain birds comes out also in reactions which lead to the hiding of food particles. In one species these reactions may take place quite openly, in the presence of other birds, and may thus be ineffective, while in another species they take place when other birds are absent, and thus will be effective. Birds, through experience, may learn to recognize dangerous instruments, such as guns during the shooting season; individuals of the same species may behave differently in city parks, where their experience has shown that they are safe, and in other zones where they are exposed to attack. It appears, then, that modifiability and individualization in behavior, in general, are greater in birds than in fishes, greater in mammals than in birds, and greater among the higher than among the more primitive mammals; in animals, it reaches its highest development in the anthropoid apes, which are, however, still much inferior to man. Thus evolution of individuality signifies an increasing variety and variability in individual reactions, a greater adaptabilty to and a greater significance of the environment; not only do the possible reactions of the individual become more numerous, but also space and time become more differentiated and they assume greater meaning in connection with the reactions of the animal; space and time become subdivided to a greater extent; they also become more individualized.

But, increasing complexity of behavior does not mean the actual loss of instincts. Essentially the same instincts are present throughout the whole vertebrate series; they are, however, associated with and, in the highest organisms, covered up by reactions in which the behavior is modified first by memories of sense impressions and by the action of suggestions; still later, by thoughts and memories of thoughts, and by the increasing significance of abstractions and new syntheses. Concomitantly, there is a decrease in the predictability of actions and attitudes of individuals. In order to make predictions it becomes necessary not only to know and to analyze the stimuli which act on or in an individual at a given moment, but also to know his past history, the stimuli which have acted in previous times and the situations which he has experienced. With the growing importance of thoughts and of the manipulation of thoughts, with the greater power to make abstractions, and syntheses, and the increasing significance of the imaginative, creative mind, the predictability diminishes, not only in regard to the actions of others, but also to the actions of ourselves. However, there is reason for assuming that not only actions appear unpredictable, but also the formation of thoughts,

psychical-social organism, together with his reactions to them, are the expression of the individuality in the sense in which this term is actually applied to higher organisms. Hence individuality is associated with the appearance of freedom in his reactions and with the increasing difficulty in establishing causal relations between the environment of the individual and his actions. This is the condition we have in mind when we speak of freedom of the will. The greater the complexity of the constellations into which the individual and his environment enter, the greater become the individual variations in actions. Thus, individuality in the psychical-social sense, the difference between the reactions of different members of the same species, the complexity of the factors determining the behavior and the non-predictability of individual responses resulting from these complexities, increase with increasing complications in structure of the organism as a whole, and especially of the nervous system.

We may summarize the essential features in which individuality in the more primitive vertebrates differs from that in the higher vertebrates as follows: (1) The stimuli which call forth a reaction are more simple and stereotyped, (2) the reactions which take place are more limited in number and are likewise more stereotyped, and (3) the degree of modifiability of the reaction as a result of previous experiences is of a lower order in these more primitive forms, and it increases with increasing complexity of structures. With ascending evolution and the more ready formation of conditioned reflexes, learning takes place more easily. Structurally, this increase in complexity and modifiability of those conditions of the behavior by which we judge individuality in the psychical-social sense, is paralleled by the development and increasing differentiation of the cortex in mammals and by the transfer of the control of the most complex reactions from the corpus striatum to the cortex. As Whitman has already pointed out, the development of instincts, which are so significant in the psychical-social life, especially of the more primitive vertebrates, corresponds to the structural development of various organ systems; both gain in complexity and in this respect take a parallel course during evolution, and it is possible to use for taxonomic purposes instincts as well as organ structures. Instincts and behavior generally are contingent on certain organ functions and they are the direct expression of organ differentials and not of organismal differentials, on which they depend only indirectly. Simple changes in texture, color of the skin or of appendages of the skin, and movements may determine species and individual reactions, and special movements and composite series of movements (ceremonies) may function as stimuli in sexual life. Identity of stimuli in several related species may cause identity of reactions of these species towards one another, at least temporarily; if later other stimuli begin to function, which differ in certain of the species, they may then call forth a differentiation in the reaction of these species towards members of their own and towards members of the strange though related species.

The complexity of the reactions and the significance of learning, of modifiability of behavior through previous experiences, seem to be in general

on a specific substratum. The regeneration of lost portions in a primitive organism is attributed to the initiating action of a psychical process corresponding to memory and thought in higher organisms. Adaptive features characteristic of a species are due to the action of a memory-like agent, and instincts are remembrances of formerly purposeful actions.

Similarly, a mneme-like agent would direct the return of certain animals at definite periods of their life to the place where they had been at a preceding phase, and the sentimental attachment of human beings to their place of birth and to their nation would be an analogous process. In like manner, the habits of social insects are compared to human social modes of living and institutions. But in the origin of the latter there enter thoughts, suggestions, and many other factors so loosely connected with each other that they appear as accidental. Human social life is modifiable. Men may even dispense with social life almost altogether and live as more or less isolated beings. Human institutions are plastic, although ultimately they also may have their roots in reflex systems, while insect organization depends almost exclusively upon the action of reflex systems which are non-plastic, fixed in character. Thus complex social phenomena, in which modifiable suggestions of various kinds and experiences in the social struggle due to variable cultural constellations play a prominent part, are considered as closely related to the reflex actions of more primitive organisms, and the hypothesis is introduced that the complex factors which are potent in human beings are likewise potent in much more primitive organisms. Instead of explaining the simple by the complex, it seems more promising as a method of investigative procedure to attempt to discover the more simple components in the complex processes and to reduce, therefore, the latter to the former. On these alternative modes of procedure seem to hinge the chief differences between mechanism and vitalism in the interpretation of living organisms.

A further assumption holds that there is a non-causal, irrational component, inaccessible to scientific analysis, in human behavior. A part which is not yet analyzed and still unknown is identified with the unknowable, and the unknowable is considered as not subject to the regularities existing elsewhere in nature and therefore as irrational from the human point of view. Support for this belief is sought in the lack of determinism which characterizes subatomic phenomena, where it is possible to determine either position or velocity of the constituents of an atom, but not both at the same time, and related to it is the assumption that because fictitious statements play a temporary role in science and because the symbols we use are only imperfect representations of reality—behaving in certain respects like metaphors with an "as if" character—all our conclusions are equally fictitious. The entelechy of Driesch would also constitute a metaphysical factor directing organisms in general as well as human personality, and even some experimental biologists, who analyze life processes in accordance with mechanistic principles postulate in addition the action of specific vital forces which are inaccessible to experimental methods.

The existence of agents other than those physical-chemical factors known

which are contingent on a variety of experiences and on conditions within the organism. Because instinctive reactions, memories and sense impressions, previous thoughts and emotions enter as constituents into the type of our behavior and the texture of our thoughts, both behavior and thoughts have become so intricate that for practical purposes they are no longer determined and predictable. Instead of predictability, there arises the appearance of free will.

In philosophical discussions there are, in its original meaning, two characteristic features associated with the concept "individuality." One assumes distinctiveness of the whole, and the second the impossibility of division without loss of the individual character. In the latter sense, a primitive animal, consisting of segments which can be separated from each other, without destroying the life and main characteristics of the organism, is less individualized than a more complex organism in which the parts are more closely knit together and in which a separation of the significant parts is not possible without destroying its individuality and even its life. The individualization of organisms has advanced the further the more integrated the parts are, so that they form one connected whole.

The bodily mechanism of the more complex organisms is unified into individual wholes especially by the individuality differentials, the nervous system, and the hormones carried by the circulating fluids. The psychical-social individuality is co-ordinated essentially by certain predominant instinctive mechanisms and by those conscious processes which center around the "I" thought complex; but this process of integration at best is imperfect and in various essential respects the psychical organism remains dissociated. But biologists and philosophers have attempted by other concepts to integrate the bodily and the psychical parts of an organism into one whole. There is the concept that a separate agent, not further accessible to analysis, dominates the parts and unifies them into a living organism; this agent is assumed to exist only in living beings and to differentiate the living from the non-living. Others attribute to the whole, new characteristic features which "emerge" in a manner not to be foreseen, if one considers merely the parts of which the organism is composed or the forms from which the organism has evolved. It is held that the new whole, in a way which is not accessible to further analysis, determines and directs the functions of the various parts and co-ordinates these functions. If a part regenerates the complete organism, it is assumed that the structural plan of the whole determines the regenerative processes. Similarly, according to this view, the end accomplished by the functioning of a system of reflexes determines the formation and mode of action of the reflexes of the functioning whole. Tacitly, thus, an agent endowed with purposeful action is introduced into the organism; it not only co-ordinates the parts but has helped to create the organism.

Another hypothesis assumes the existence of a "mneme" as the agent unifying the parts of the organisms. The memory of a preceding change alters the future state and behavior of an organism in a specific manner, which is conditioned by the character of the first change or experience acting

Chapter 2

Individuality and World

In the preceding chapter we have followed in the animal series the evolution of individuality in the psychical-social sense and we have seen that it reaches its full expression only in man. This highest type of individuality we shall now analyze still further. The term "individuality" implies a distinction between the organism with its psychical attributes and activities, the inner world, and the surrounding, non-living, as well as the living and human social world, the outer world. It also suggests the concept of the uniqueness of the individual and of his self-determination in his relations with the environment, in contradistinction to the organism as a mechanism or an automaton; self-determination carries with it, as a corollary, responsibility for one's actions and attitudes. These concepts of individuality have arisen in the course of the activities of daily life, in response to the problems man has to face and the manifold difficulties he has to overcome. For a fuller understanding of the development of the feeling of individuality it will therefore be necessary to analyze the distinction between inner and outer world.

On the basis of our sense impressions and by means of abstraction and synthesis, we have created a thought structure of the surrounding non-living physical and chemical world, as well as of the surrounding living world of organisms, and in both worlds the same constituents occur. The environmental factors act on our senses as stimuli and may appear to us partly as variable and partly as constant factors, while we assume that our sense organs are constant, although they also in reality may be variable. After we have dissociated from ourselves the outer non-living and living world which, by means of critical analysis, we have transformed or attempted to transform into constant and variable physical-chemical units, we consider further the interactions of the outer world with our sense organs, nervous system and other constituent parts of our body. In this analysis we may tentatively regard the elements of which the outer world is composed as more or less constant and our body and its constituents as variable. Through the study of the variability of our organism in its interaction with the environment, we create the science of physiology. As a result of this interaction between the outer world and our own organism, there develop on the basis of our sense impressions thoughts and emotions, which on their part may then interact with our nervous mechanisms, with our muscles and our bodily functions. There is thus a circuit from the outer world by way of sense impressions and our organ systems to thoughts and emotions, and from these, in the reverse way, to the outer world. This circuit we study by means of abstractions that are shifted and re-synthesized in such a manner that the essential constants are separated from variable, accidental factors; such an analysis,

to be active in both the inorganic world and in organisms, might serve not only to unify the living individual into one indivisible whole, but might also imbue him with a distinctiveness which is one of the implied characteristics especially of human individuality. It would therefore satisfy a deeply felt desire of man to be "himself" only, to be unique and endowed with self determination and free will. Yet the investigator must proceed in the study of individuality according to the rules which alone have proven successful so far in all the other fields of science.

of our inner world are variable; they cannot apparently be referred to environmental constants with the same fixity and definiteness as can the sense impressions; thoughts and emotions seem individual and indeterminate, differing in different persons at the same time and place. Hence, thoughts and emotions appear not to be predictable.

Thoughts represent abstractions and syntheses into which memories of individually varying experiences enter. The emotional reactions also vary widely in different persons and have much of the character of a mystery, because in the individual affected they are largely unanalyzed. In this sense our bodily organismal constitution as a whole, and especially the brain activities, in which our thoughts and emotions originate, seem more particularly our own than the operations of the sense organs, which reproduce for us our environment. What is unique and unexplainable, and therefore apparently free and not a directly determined function of the environment, we refer thus to the inner world, to our psychical individuality.

But if we analyze our inner world still further, we find, as stated above, that it as well as our outer world consists largely of sense impressions; these enter as essential constituents into our pictures, thoughts and wills, which are derived primarily from the outside. There may also participate in the construction of our inner world, those sensations which originate in certain parts of our own body. When we speak our thoughts or see our own body, we perceive them through the ear and eye as we perceive those of another individual. Certain psychologists go so far as to maintain that all our thoughts are perceived as the result of the activities of our speech muscles, even if we do not actually speak. It is especially the memory and anticipation of the feelings associated with muscle contractions in response to certain thoughts and pictures which make us aware of our will. Furthermore, there is added to the central, psychical constituent of our individuality, a picture of our bodily configuration. We acquire this picture of our body gradually as we acquire that of another individual. Our body, as well as our mind, is to a large extent strange to us. If we see our own image in an unusual set of mirrors, we are astonished that this is a reflection of ourselves. The physicist and philosopher, Ernst Mach, when entering a bus and seeing his image in a mirror exclaimed, "Who is the school teacher that enters this car!" We have to become acquainted with our own body as with the body of another person; also with our own mind as with the minds of others. The "self" or "I" is an abstraction; it does not really exist in the sense in which we believe it exists. We hardly know our own self any better than we know other individuals or the world around us.

However, when we analyze more in detail our individuality in the psychical sense, we find that the interactions between what we consider as outer and as inner world are still more complex than the preceding considerations have indicated. This is due to the fact that the thought reflex works in two directions. A thought as a representation of the outer world may set in motion in our organism corresponding functions of various organ systems, and in particular, motor reactions; thus the picture of a good meal

as far as sense impressions, the origin of thoughts and emotions and their effects are involved, represent the subject matter of psychology. As a further step we recognize in our outer world other human organisms, consisting of physiological and psychological factors similar to our own, while other living organisms—animals and plants—show graded differences from ourselves. The physical-chemical, non-living environment, as well as the world of living organisms, except ourselves, represents then our outer world, while we, with our sense impressions, feelings, thoughts, emotions, wills and desires represent our inner world.

Thus our inner and outer worlds both consist to a large extent of the same psychical elements, our sensations; also, both worlds are composed of the same physical-chemical elements and this is true of all organs and tissues of the body, including those on whose functions our psychical activities depend. In the physical and psychical realms, the outer world and "we" are constituted of the same elements. Sense impressions stand on the borderline between inner and outer world, and they, with our thoughts, represent a combination of environmental and inner organismal factors. It is by means of sense impressions that we construct both worlds and connect the two. With their aid we build an outer world, to which we attribute an existence independent of our own organism as a separate external reality, which we take for proven because of the fact that our interpretation of things and our prediction of future events, made on the basis of our thought-constructions, may prove correct, and also because of the fact that this reality is experienced in the same way by all human beings who have adequate knowledge and understanding.

We believe that we are aware of our inner world directly, without the intervention of our sense organs, while we realize that we recognize the outer world by means of these sense organs. The inner world constitutes for us our real individuality, and especially those parts of our inner world centering around the concept "I," which latter again is gained by means of abstractions and synthesis, like other thoughts. As far as our psychical elements undergo within us variations which we do not fully understand, and which are different at different times and which may differ in different individuals under apparently the same conditions, they are considered as subjective. The outer world is considered as objective, independent of changes within us, and to a higher degree constant. This is one of the reasons why we make such distinctions as outer and inner world.

Through the interaction between the outer world and our sense organs we become aware of events, which are singled out and differentiated from others and compared with similar preceding ones. Events then become predictable; the sense organs appear, thus, to be constant, identical at different times in the same person in the same environment, and also identical in different persons; they seem largely independent of other parts of our variable organism. Those sense experiences which are common to all humans, which are reproducible and which represent, therefore, a mechanism, we tend to refer to the outer world. On the other hand, thoughts and emotions

ducible, not explainable; hence individuality is assumed to be essentially non-rational. But in reality, the nature of individuality represents a problem to be analyzed and explained. The non-rational of today may be the mechanism of tomorrow. The manifoldness of human individuality depends upon variations in the organization of the individual and in the reactions of the individual to different environments. These are accessible to analysis and there are at least indications that such variations are the manifestations of connected mechanisms.

Our will is assumed by us to depend on our thoughts and inasmuch as thoughts appear as isolated phenomena, detached from the reflex circuit of which they are really a part, and inasmuch as we have forgotten the experiences which gave origin to them, our will appears to us as a free, indetermined phenomenon. It is not predictable, it cannot be duplicated in others, it is considered by us to be our own, the expression of our individuality, which is therefore characterized by free will.

Yet the more we study the actions and attitudes of human beings, the greater the degree of our experience, the less becomes the range of indetermined actions. We learn to know of the reflexes active in us and of the establishment of conditioned reflexes; we analyze our sense impressions, our thoughts, which depend upon sense impressions and complex experiences; we observe the accompaniment by emotions of thoughts and motor activity, and in particular of inhibited activities; and furthermore, we note the stimulating and inhibiting effects of suggestions, those given from the outside by the spoken words and actions of others and those resulting from our own thoughts and actions, which we remember as we do those of others and which function in like manner. Especially susceptible to analysis are experimental posthypnotic suggestions and their consequences, which subjectively may appear as expressions of free will. Furthermore as others issue commands to us, so we issue commands to ourselves We know what, under certain conditions, will happen to our person, as we know what will happen to things and living beings around us. Remembering the consequences of former experiences and the thoughts and actions following our choices and decisions, new, complex conditioned reflexes, pleasant in some cases, inhibiting, painful in others, develop and complicate our attitudes. The choices and decisions made in the past, act therefore as suggestions tending to influence our future course. But in addition, we know of the effect of chemical and physical factors and of changes in our bodily structures and functions on our thoughts and actions. All our experiences and the subsequent analysis of the interaction between our thoughts and our organism affect and regulate our bodily reactions. This very complex, and therefore incompletely known and only partly predictable balancing of factors which determines our actions, is what is felt as freedom of will in the circuit of outer world → sense impression → thought → reaction. Usually we develop [illegible]

the feeling of inner freedom. These relations of "ourselves" to "ourselves,"

stimulates gastric secretion and certain pictures with sexual content may set in motion, by way of reflex, certain sexual activities. These pictures may be supplied by social institutions created by us, as for instance, by eating places, by theatres. The outer world thus interacts with our organismal functions by means of pictures, thoughts. But, conversely, gastric contractions during hunger may stimulate certain picture-thoughts of a good meal, with the accompanying emotions. Or certain reflex processes occurring in the sexual organs may secondarily call forth the corresponding thought-emotion complexes, and the memories of the latter may subsequently again set in motion sexual functions. In this manner a very intricate play between outer world and inner world, between our thoughts and our organism, constantly takes place. The outer world acts on our inner world by means of sensations, pictures and thoughts, which also function as suggestions, and through them the outer world influences our actions and our attitudes. Thus, thoughts having their source in sense impressions, exercise their effects essentially through the things and events which they represent, and there exist only quantitative differences in vividness and effectiveness between the direct experience and the effect of thoughts; both are complicated, but to a varying degree, by a relationship to other thoughts.

Furthermore, those of our motor reactions which follow thought-emotion complexes and are often induced by the latter, may lead to thoughts which are conscious and which make connection with the "I" concept, while the simple reflexes connecting our senses with our muscles by way of ganglia, are usually unaccompanied by conscious thoughts. There are, besides, many sense impressions acting on an organism, together with memories of past experiences, which do not find direct release in motor actions but merely in thoughts and emotions; these again tend to lead to an extension of conscious thought and emotion processes; they may ultimately find expression in scientific, philosophic, or artistic productions. In order to be able to understand and predict the phenomena of our varied reactions, there would be required in all these cases, a much more intricate and searching analysis of the common factors underlying these processes, than can, as a rule, be made at the present time.

Processes and things involving common unit factors and differing from others in experimentally reproducible constellations of these unit factors and their mutual relations are mechanisms. In general, constant relations between events, which show definite sequences in time, are what we consider causes and effects, the former preceding the latter constellations; to establish these relationships is to explain; what can be explained in this way is in a wider sense a mechanism; it is opposed to what is indeterminable and non-rational. However, in many instances we are satisfied with attaching a word or a label to a thing or process, and having thus attained the possibility of handling in our mind this thing or process for the purposes of mental operations, especially in accordance with the requirements of the natural and social struggle, we are satisfied. In contrast to the term "mechanism" "individuality" implies, by definition, something unique and therefore not repro-

thoughts, are sources of our pleasure and pain, and we treat thoughts as we do complex sensations; we divide them into parts, eliminate some of these and synthesize others, and again the process of creating these abstractions gives us the impression of free will; it appears to us as individual, as an action carried out in accordance with "our" wishes, in contrast with the rigidity of a direct sense impression → motor reaction chain—where the reflex mechanism is much more evident. Our ability to combine series of thoughts into into one associated texture, with which process is joined the memory of thoughts and their subsequent realization in actions and the connection of these thought textures and actions with the "I" are functions which represent the highest degree of consciousness.

What we experience, then, above all, as our freedom and as the expression of our individuality, is this ready formation of picture and thought textures of a coordinated and of a superordinated kind, the latter representing a more comprehensive abstraction and synthesis of thoughts.

But observation and analysis reveal to us that there are many limitations to our apparent freedom. There are, above all, suggestions which limit the free association of our thoughts, limit thereby our freedom, the expression of our individuality; however, this lack of freedom is not always conscious, it is recognized by means of superordinated thoughts only under certain conditions, more especially if the suggestion takes the form of a command imposed upon us and thus conflicts with the spontaneous trend of our thoughts. Also, fashion, ritual, tradition, which are systematized suggestions often functioning as habits, and the suggestions given us in childhood may limit the freedom of our actions, the expression of our rational activity; but these inhibitions and limitations even more commonly may not become conscious in us, because as habits they do not usually lead to inner friction and conflict, but rather give us mild and pleasant emotions, and function as normal constituents of our psychical life. It is only those suggestions which strongly disturb our systems of thoughts and wills, which become conscious in us as outside interferences, against which we react. Other suggestions, on the contrary, may give us emotions of a very satisfactory kind; they may sustain and justify our thoughts and wills and support us under adverse conditions. We justify and uphold then such suggestions and we react emotionally against thoughts or conditions which tend to oppose them and to prevent their realization.

However, even suggestions which are accepted as a part of our own thought-system and which act unconsciously may restrict the freedom of our thoughts and actions and have far-reaching effects, inasmuch as they may limit our contact and our relations with the social and non-social environment, the growth of our individuality, and our ability to discover things and to exert rational self-control. They may interfere with the processes from which result recognition of new elements in our environment and a more adequate adaptation to the environment. They may thus tend to diminish the horizon and content of our world. In particular, they may restrict the deeper understanding of the social struggle and the corresponding develop-

the effect of our own thoughts and suggestions on our behavior and attitudes, and especially the fact that the addition or lack of a thought may turn the balance in our responses in one or the other direction, all these factors constitute largely the substratum which, in its complexity, gives us the feeling of freedom; and it is just this apparently free and non-determined, or rather self-determining, part of our actions and expressions which we feel as the most characteristic feature of our individuality.

The most conscious thoughts associating readily with other thought-emotion complexes, and especially also with the "I" complex, the directing thought-emotion processes in us are felt as the constants in our individuality, which operate and connect the states of our changing organism and our actions in successive periods and apparently make it one homogeneous consistent whole representing our real self. It is this part of us which seems to us to be independent of the outer world, in contrast with our bodily functions and simpler nervous automatisms, which evidently depend upon the interaction with the outer world and which do not therefore represent solely ourselves.

The central governing thought-emotion processes, affect in a direct manner our own organism, and in particular our muscles, but secondarily they can also affect and change our environment, of which, to some extent, we thus become the master. In a measure however, we can, besides, control ourselves. Our conscious thoughts can automatically, by their mere functioning, suppress injurious emotional reactions and direct our responses in a rational way. Thinking, as such, about our actions may thus function in an automatic way as a moderator, and it is especially through such a mechanism that we feel our will is free.

But this mechanism needs further analysis. The conditioned reflexes active in us are often associated with thoughts and pictures which we may describe and analyze and thus reproduce. These thoughts and pictures may develop in us also in a more complex roundabout way by means of chains of thoughts which, however, usually have likewise been set in motion through outside stimuli. They are of the same kind as those which are transmitted to us by others, or by reading. Thoughts may thus come to us in various ways. However, if, as usual, the source of these thoughts is not clear to us, then they appear to us to originate spontaneously in ourselves and to be the expression of our free will, of our individuality. The pictures and thoughts, associated with what we do and entering as a factor into our conditioned reflex mechanisms, may *intensify* the reflex action automatically; on the other hand, also, a process inhibiting these pictures and thoughts and at the same time making the thoughts conscious may develop, and this latter process may interfere not only with these pictures and thoughts, but also with the primary conditioned reflexes which set these processes of thinking in motion. In this manner rationally connected thoughts may interfere with our primary, more simple reactions; they may control and make rational our actions, and they exert these effects by means of mechanisms which may not become conscious to us. Complex sense impressions, their memories, as well as

personality. This is the world of the social struggle and the struggle with nature.

Poetry and art have been, and continue to be, largely expressions of our egocentric attitude towards the world; they represent us and our experiences, our feelings and emotions. They attach meaning to our world, or they intensify and extend the meaning and significance of our world and of our life and they may picture a world and life from which fear and disharmony are more and more eliminated. They tend to convert an inanimate, cold, non-feeling world into an animate world, in which feeling and human meaning is extended and intensified, so that we can find ourselves and beings like us everywhere. But gradually the character of this egocentric world has undergone changes. The surrounding world, the universe, begins to center around us in a different sense. In the interactions between the outer world and ourselves we find identities and differences; we abstract from the differences and combine new similarities. In this way there is gradually created a second world, that of simple and complex sensations and a logical world of things and interactions in which we and beings like ourselves live. It no longer centers around ourselves and in it we are merely a small part of a strange universe; but more and more it becomes to us the real world; it is the world revealed by scientific analysis and synthesis. We adapt ourselves also to this world; we make it our own by understanding it and we attempt to make it respond to our needs, wishes, hopes and fears. There still radiate from ourselves thoughts and emotions out to the universe; the universe is still bound up with us and we with it in one thought structure.

In the course of time there begins to be added to this analysis of the outer world, an analysis of our inner world, of the world of myth which we have created, of social and natural struggles and the emotions they elicit. We form concepts not only of the environment and of other human beings, but also or our own organism or parts of it and its relations with the outer world, and we realize the vast differences between the egocentric and the non-egocentric conceptions of the universe. These two developments, that of the egocentric world, eventuating in the formation of pictures of ourselves around which everything else centers, and that of the objective world, of which we are merely a very small and relatively unimportant part, have proceeded side by side throughout human history. It is the varying relative importance of these two world conceptions which have determined largely the nature of our civilization, and the life we live is a compromise between these two antagonistic attitudes.

Yet physiologically each of us remains bound up with his organism, and the needs and functions of the latter continue to make ourselves the center of our own small universe; we therefore still make use of poetry and art to change the concept of the world around us and of human beings and human relations in accordance with our needs and wishes. It is the analytic, scientific world which restricts such egocentric thinking and influences and changes the values we attach to things and the laws we make. Science creates

ment of sympathy and pity. Thus, rigid ritualized group-action may replace our initiative and coordination of thoughts and actions as the highest expression of our individuality. On the other hand, these types of organized suggestion may also be helpful; they are economic, inasmuch as they save us the expense of energy in the process of analytic thinking and of creating; and moreover, they provide the feeling of security and remove from us the weight of responsibility. One of the most distinguishing features between different individuals is the relative power of suggestion, on the one hand, and of freely associating and directing thoughts, on the other. Furthermore, the tendency of these thoughts and their resulting actions to make conscious connections with the "I" and the extent and comprehensiveness of the "I" differ very much in different individuals, the different "I's" varying greatly as to their content of environmental constituents, especially those of a social character. The "I" concept is already present in the young child; it may be active also in our sleep, where we refer to and connect with "ourselves" memories usually of recent events, of thoughts we have had or about which we have read.

The more readily we remember our thoughts and consequent actions, and the more we are capable of relating them in a consistent and logical manner to the whole texture of our thoughts, and especially to the "I" concept, the more our thoughts and actions are modifiable. On the contrary, in so far as thoughts function as suggestions in us, they are rigid and our actions and attitudes are not readily modifiable. As stated, individuals manifest marked differences as to the relative preponderance of modifiable and less modifiable thoughts, as to their sensitiveness towards thoughts and impressions coming from the social as well as the non-social environment, and as to the readiness with which thoughts and impressions act as suggestions. The easily remembered conscious thoughts are relatively labile, while the unremembered thoughts, suggestions, or the processes underlying or accompanying them, influence our actions and attitudes in a rigid manner because they are separate and not readily brought into connection with other thoughts.

The relations between inner and outer world have changed in our consciousness in the course of human history. Man created pictures of an outer world and of an inner world; in so doing he created not only an anthropocentric world, but also an egocentric world. He saw himself, or beings like himself, everywhere in the outer world. He felt the fate of the others as his own fate and their experiences called forth emotions of sympathy and pity. Or he reacted to them as to competitors or enemies with emotions of dislike and antagonism. He obeyed or struggled against other human beings who gave him commands. The effects of suggestion predominated very much over the purely logical-intellectual analysis; the emotions of fear and hope were correspondingly very active. His world centered around himself. In this egocentric world things have values as material goods or as psychical goods. Men fight for these, for the maintenance and elevation of their individuality and for a certain picture concept which they have of their own

but the knowledge thus gained is used in the social and natural struggle, not only for helpful but also for destructive purposes. Similarly, biological concepts are employed not only for the alleviation of the cruelties of the social and natural struggle, but also as weapons in the social struggle, which serve to aggravate the latter and to introduce into it added cruelties and to intensify the unbalance of the mind as well as of the body. Thus the science of genetics in its applied form as eugenics has been, in certain instances, used by scientists themselves, as well as by others, in the interest of nationalistic tendencies in a struggle for distinctive psychical class goods and for economic advantages.

The same tendency reveals itself in still other ways. There are certain concepts which play a great role in the social struggle and in the adaptation to the painful realities of the natural struggle. Man constructed in early times the thought of something which is not subject to the sorrows and destruction that we experience in actual life, a spirit within us whose expression is free will. Since free will involves intention on the part not only of ourselves but also of the other individual, the exercise of it may affect most keenly the social struggle; whether we consider an act as hostile or not depends in many cases not so much on the act itself, as upon the intention which inspired it. The idea of intention, furthermore, is intimately connected with that of responsibility, hence we mete out gifts to one who is helpful and virtuous, and punishment to an offender. These thought-constructions represent an adjustment, by means of which we uphold our individuality in its more primitive needs, but at the same time they may lead to cruel repressions or to undue elevation, effects which often aggravate the social struggle. Into such a mental environment of the egocentric world the scientist is born, as are other human beings, and he, too, often upholds these concepts which are active in him as suggestions. On the other hand, the application of the concepts of science, based on an increasing understanding of the springs of human behavior, tends to substitute understanding, prevention and cure for punishment and suppression, and thus to mitigate the harshness of the social struggle.

Human life, then, may be considered essentially as a struggle with nature and as a struggle with other human beings—a natural and a social struggle. The natural struggle is a struggle for the maintenance of our organism, which is so constructed that it gradually deteriorates, ceases ultimately to function and dies. It concerns itself with the satisfaction of material needs in an environment to which we are only incompletely adapted and which only by degrees we learn to know. In certain respects there is an antagonism between us and nature, which ends with our destruction. At the same time, we interact with other human beings and in this interaction a complex social structure has been built up; to this also we are insufficiently adapted. Thus the struggle for our preservation, which under more primitive conditions was largely a struggle with nature, becomes, over a wide range of life, a competitive social struggle for material goods, and there is added to this, more and more, a struggle for psychical goods. This struggle for psychical goods,

new symbols for the manipulation of things and events and for the understanding of reality. The new thought constellations, including those concerned with our own person, are more and more removed from the world in which we directly live and feel pain and satisfaction, the world of the natural and social struggle. But the latter also forms a part of our analysis; our experiences are split by us into parts and these parts are shifted and similar parts synthesized into new concepts, which, as abstractions, become more and more removed from the original direct experiences. This thought world is therefore different from the directly experienced world; it is a re-organized, a differently and a better ordered world, which allows us to a certain extent to understand and to master the world of direct experience. The concepts thus created are devices allowing us to orient ourselves under new conditions, without undergoing again all the manifold experiences for which the concepts stand. Science functions in an objective thought world, which is less emotion-tinged, less and less actively involved in the various phases of the social struggle and in the particular desires of our individuality. It is the world in which also the dominating factors of the egocentric world are studied as to their origin and nature; psychical goods and material goods become here objects of analysis and synthesis. Imagination and its creations in poetry and art are likewise objects of examination and our particular individuality recedes in importance, except that it continues to function as the analyzing and synthesizing, and thus as the scientifically creative agent. Yet, we can use the symbols thus created in modifying the frictions of the egocentric world in an effective way; the cruelties of the natural and social struggle may become more and more mitigated and the individuality secondarily gains in value on a more realistic foundation. Thus, by means of science we may, within a certain range, learn to dominate our organism as well as our environment.

The scientist enjoys his creative work, plays with his thought symbols, just as the poet, artist and musician play with imaginative thoughts, colors, shapes and sounds; they all abstract from the whole reality as it is directly experienced and select only certain parts of the latter. The poet, artist and musician create things that are meant to supply and maintain or elevate directly or indirectly the value of psychical goods in the natural and social struggle, and thus to sustain and elevate the struggling and suffering individual. But the scientist, playing likewise, creates a thing that becomes his master, is independent of the direct, primitive experience of the individual and of his struggles. It dominates the investigator, who finds himself more and more limited by his own creation, the thought structure, which is science. His erection of these thought structures represents a vital process, in which imagination is an important instrument, yet which, as far as the influence of the created concepts reaches, restricts his imagination; it limits him in shaping his life, his picture of reality in accordance with his wishes and in accordance with his imagination. However, even into the building up of science egocentric tendencies penetrate. The analysis of those elements of which the outer world is constituted makes possible a mastery of this world;

of other organ systems. In the end, the stunting of personalities thus initiated reacts also unfavorably on those who have been responsible for these effects. Certain nationalistic, racial and social caste-distinctive psychical class goods are the most prominent types of these injurious agents, in the production of which there cooperate human inventiveness, the accidents of history and the desire for psychical self-maintenance, for elevation of the personality picture, for mental security and for compensation for injuries received in the social struggle. Humanity stumbled on these social instruments, as it did on some of the basic stimulants and narcotics; those who had the power made use of them, found them to their taste, and their use became general.

These functions and consequences, which apply clearly to distinctive psychical class goods, apply more or less to all distinctive psychical goods, although their injurious effect is probably greatest in the case of distinctive psychical class goods. There are other types of distinctive psychical goods that are more individual and less injurious, such as recognition, fame and glorification of a person who excels in ability and creative work. Warriors, statesmen, philosophers, scientists and artists wish to have the benefit of this type of distinctive psychical goods, which in addition to other advantages provides a certain type of immortality and thus promises compensation for the inevitable defeat in the natural struggle.

Ultimately all psychical goods—simple and distinctive—which we receive from the outside become converted into and contribute to the creation of inner psychical goods, of thoughts and emotion complexes, which we cultivate and which sustain us in the social and natural struggle. Art, philosophy, religion, science, principles in general, serve as the objective material of these inner psychical goods and, consciously or unconsciously, they center in us around the "I" concept. From the latter there develops more or less clearly in every person a personality picture of himself, which is perhaps his most precious possession. If this is on a high level and unchallenged, the whole organism functions relatively well; if it is questioned, attacked and lowered, serious consequences for the wellbeing, mental and physical, of the individual may follow. Thus the preservation and elevation of inner psychical goods, more or less centering around a personality picture, become the main objective and the principal agent in the social struggle of individuals with one another, and one of the most common weapons used in this struggle is a suggestion, often attached to a word, thrown into the system of inner psychical goods of an individual, which acts as an incompatible agent and which tends to distort and lower his personality. In different individuals the character of the inner psychical goods varies, in the same sense as do their convictions, the prominence and potency of their principles, the simple and distinctive psychical goods which they have received, and their inherent ability to face the difficulties of the social and natural struggle. Suggestions which have been given to human beings, often in the early years of their life, determine largely the character of their inner psychical goods and their personality picture. Inner psychical goods have ultimately the function of steadying the individual in his social and natural struggle; and in accordance with the de-

however, is closely associated with the struggle for material goods; it also affects our organism in its most vital functions and is, therefore, as is the struggle for material goods, ultimately a contest for the upholding of our individuality in both its bodily and psychical aspects.

Our individuality requires consideration and respect; it needs appreciation, friendliness, friendship and love. These are primary needs, which provide a favorable mental medium in which we can function and develop without fears and inhibitions, and in which certain fundamental requirements in social intercourse are satisfied. We may call the means by which this is accomplished simple psychical goods. On the whole, there is the possibility of taking care of such general and basic needs of all, and the satisfaction of the requirements of one should not exclude the others from receiving their share. We can be friendly, courteous and understanding to everyone. However, some distinction sets in even here. We cannot give friendship and love to everyone to an equal extent. Certain individual distinctions are made; but if they are associated with the giving of the more common simple psychical goods to all, with understanding and appreciation of all others, and if the latter also can be supplied by those nearest to them with the needed individual psychical goods in the form of friendship and love—which in a measure partake of the character of what might be called individual distinctive psychical goods—no injury should result. Still, even then the psychical balance may be imperfect and the giving or withholding of such individual distinctive psychical goods may, in many cases, lead to the bitterest struggles, even within the same family; intense mother-love may become so exclusive that it leads to bitter jealousy and to severe antagonism towards those with whom she has to share the love of her children.

Of still greater significance perhaps as the source of severe social struggle are the distinctive psychical class goods; the latter usually appear as social caste spirit, family and race pride, and nationalism. These lead to destructive struggles of a political, economic and social character. They may end in war and revolution. It is of interest that the struggle for these distinctive psychical class goods is usually associated with a struggle for material goods; these two types are intimately connected, material goods being, to a certain degree, valued for the sake of the distinctive psychical goods they provide, while the possession of psychical goods often gives ready access to the acquisition of material goods. Among the distinctive psychical class goods, those have an especially devastating effect which make the possibility of acquiring these goods a constitutional, inherited condition in certain classes of human society. Under these circumstances no hope of improvement is conceivable. Moreover, the gain in distinctive psychical goods in some is predicated on the lack of them in others, since if all possessed them they would lose their significance as distinctive psychical goods. The effects on these others of social humiliation, which is the consequence of the social recognition of distinctive psychical class goods, are very injurious. They cause directly a serious interference with muscular coordination, with expression, initiative, controlled imagination and action, and indirectly, an interference also with the functions

this objective struggle, in which important work of value to humanity is done, the subjective struggle, which aims at material goods, not for the whole human society but for the individual and his family, and at distinctive psychical goods. This second struggle is largely, although not altogether, a competitive one for a position, for profits in the realm of material goods, and for distinctive psychical goods yielding recognition, distinction, honor for the individual and those he represents. From a certain aspect, the struggle for distinctive psychical goods might be considered as a competitive struggle for profit in the sphere of psychical goods; but it is not designated as such, because while the profit motive is approved by public morality in the sphere of material goods, it is regarded objectionable in the sphere of psychical goods; here, the aims should solely be objective. But if we analyze human activities, we find present in all of them the objective and the subjective motives. This is true of the life of individuals pursuing commerce and industry, as well as of those pursuing pure and applied science and art. However, these two motives are present in varying proportions in different occupations and in different individuals.

It is, above all, the manner in which this subjective struggle is conducted which characterizes individuals. All the psychical characteristics and the corresponding modes of reaction which distinguish one human being from another may be called his personality, and it is especially in the subjective social struggle that the personality becomes manifest. There is still another motive which may participate in this subjective aspect of human endeavor; this involves the desire to be distinct from others, to be an individual in the true sense of the word, particularly in the psychical field, in thinking and feeling, and in creating; it is accompanied by the wish not to imitate others, but to express one's own individuality, to receive recognition for this distinctiveness, and to be accepted as an individual in one's own right. The degree of self-control and self-maintenance and determination which an individual exhibits in the social struggle is a measure of his "morale." It represents his ability to resist the results of injurious suggestions which tend to disorganize his personality, depress his self respect, the feeling of his strength and his ability to maintain himself and to be respected by others and by himself.

To harmonize the various conflicts of individuals and groups in the social struggle certain codes have been established, Ethics and law have significance as means of such an adjustment. They represent balances that compare and weigh two or more contrary claims or needs, but these decisions, although generalized, are as yet very imperfect, because they cannot very well include in their comparisons and weighings the different individualities around which the needs and claims center. Ethics, with its concepts of justice and of the dignity of the individual, includes in its consideration also the sphere of psychical goods, while law, with its more formal concept of justice, preponderantly limits itself to the sphere of material goods and bodily injuries, where comparisons and weighings can be made more readily in an objective manner than in the sphere of the more individualized psychical goods. In

mands arising in the contingencies of these struggles he may alter and shape his inner psychical goods, add to them or subtract from them, and modify *his* personality picture. The opportunist or politician readily sells certain inner psychical goods, his convictions, principles, for the advantages of material and distinctive psychical goods. If the personality picture, and in particular the "I" around which it centers, becomes unduly prominent in all social manifestations, an individual is judged to be vain. Very often this is a reaction of a compensatory character in one in whom social injuries make the personality picture very conscious and prominent. Related to these processes is the self-consciousness frequently associated with painful discoordination, also the result of social injuries experienced by sensitive individuals.

While, thus, simple psychical goods are needed for the maintenance of the bodily and psychical organism in a healthy state, it is especially the individual distinctive psychical goods received from and given to others, and above all, the inner psychical goods that differentiate one individual from another, which individualize human beings in the highest degree; on the other hand, distinctive psychical class goods, even when they serve as a source of self-elevation and of security and strength for one's own individuality, as a rule tend to submerge the individual, making of him a mere representative of a certain group.

Such are the main factors underlying the social struggle; and they also are its objectives. Which of these objectives occupies a pivotal position in a certain constellation or phase in the life of a person depends to a considerable extent, although not entirely, on accidents, on suggestions received. This is the foundation on which our choices and decisions are made. Material goods are so important a factor in this social struggle, as far as it concerns the attitudes and actions of wider groups, largely because they satisfy the most urgent needs and also because they are the least individualized and can, therefore, most readily unite people of the most divergent kind. Psychical goods are much more individualized and therefore can less readily lead to mass movements; but they have done so in the religious wars of the past and they often do so even now, as, for instance, in the nationalistic struggles, and when originators and leaders of political mass movements are spurred on by certain constellations of inner psychical goods or by psychical injuries received, or by an overwhelming desire for power and distinction rather than by a desire for material goods.

The objectives of the natural and social struggle affect the daily life of every individual and in a twofold way; in all his efforts his desire for material and psychical goods, and in particular also for distinctive psychical goods, enters as an important motive. Thus, in general, human beings carry on two kinds of activities, one objective, the other subjective. Their objective activity tends to increase the available amount of material goods and to contribute new values to the psychical reservoir from which humans obtain what is best in their inner psychical goods; this is an activity which tends to increase the health of body and soul in the life of an individual and of the group. In this manner the values of science and ethics are created. There is added to

tendencies, principles; between such incompatibilities he is constantly balancing, at one time one, and at another time another of these factors predominating. Our feeling of freedom and self-determination depends upon this finely balanced system of thoughts with which we adjust ourselves to conditions in an ever-changing environment. It depends, too, upon a certain continuity in thought; the thoughts of one moment must be remembered in the following period and must manifest a certain degree of consistency. We may lose the feeling of freedom in the case of ourselves and of others as soon as this finely balanced, connected system of thoughts and emotions is interfered with, owing to abnormalities in the functioning of the organism.

Thus, although the lack of freedom and the automatic character of human behavior may be evident to us under certain conditions, for the most part, we largely ignore the factors determining our reactions, and they are indeed mostly unknown to us. We live, think and act in accordance with the requirements of the situations which we meet. The interactions between the situations and our organism and our responses remain largely unanalyzed. Even if we should be aware of them, as a rule we abstract from this knowledge in the process of living. Here we feel we are free.

2. *Continuity and consistency in individuality.* We need not only the feeling of inner freedom and self-determination, we also need a feeling of continuity and self-consistency. These needs are intimately connected with each other and they both depend upon the interlocking of thoughts, the uninterruptedness of memory which joins the experience of one time-unit with those of the following, which creates a connected texture of remembered sensations, feelings, thoughts and wills.

In our changing environment, amidst the varying conditions under which we live, we have principles which as such remain fixed, in contrast with the shifting manifestations and expressions of these principles. We have conscious thoughts which direct us in our aims and we have memories of ourselves. When we become aware of abnormal, irrational reactions within us, we try to make them accord with our directing thoughts and principles. These latter attempt to co-ordinate all our thoughts into one consistent whole, which centers around the "I." Our individuality is conceived of as being more than merely a peculiarly constituted mosaic of factors, all of which may exist also in others, although in different arrangements and with different degrees of intensity; the picture of ourselves as a coordinated, rational personality becomes fixed in our mind, notwithstanding the changes which take place in our body and in our thoughts continually. Thus we live in a world of illusion, since in reality we are subject to a continuous change. Furthermore our distinctions as to what is our own and derived from the inside and what is derived from our environment are quite generally erroneous. We attribute to things within us, to our individuality, what is really of external origin, such as the suggestions which have acted on us and influenced our behavior. Quite commonly we believe that reflex actions, having their beginning outside of us, originate within us if the afferent parts of the reflex arc remain hidden from us. Thus we may hold ourselves responsible for actions of an automatic char-

particular, such terms as egoism, altruism, have a meaning only in the context of the social and natural struggle; they signify certain attitudes, balancings between our needs and those of others in these struggles. It is largely in such a world of varying conflicts that the individual lives and his activities take their course.

This world of the social and natural struggle is essentially the egocentric world, from which, step by step, the objective world of science has detached itself in the past and will continue to detach itself in the future. It is only if we consider the different psychical states active in the sphere of the social and natural struggle as this struggle has developed in the course of human history that we understand some of the characteristic desires and needs of our individuality, as manifested by our wish to attain an absolute significance and an independence of time and space. To accomplish these aims the individual longs (1) to be free and self-determining; (2) to be unique and constant, essentially unchangeable, a self-conscious continuity; (3) to be eternal, and (4) to obtain appreciation and self-justification in the face of attacks and criticism, to prove worthy of existence and to be in harmony with the laws of man and of the universe. Let us examine such needs and desires and state to what degree they may rest on constants in the human constitution finding expression in the present social constellations and how far they may be founded on illusions.

1. *Free will and self-determination.* The concept of psychical individuality implies, as we have seen, the feeling of freedom, the existence and manifestation of a self-determining entity, which according to the belief of many assumes the character of a spirit or soul, which is an eternal factor; this "self" is distinct in each person and sharply differentiated from the processes underlying our machine-like automatisms, and it presupposes the action of a directing principle coming from the inside, rather than a mechanism dependent upon the interaction between organism and environment by way of definite reflexes acting in preformed channels.

That there are fixed factors determining our actions we have already discussed; in certain cases the automatisms active in thinking and in the manifestation of emotions become so evident that, under these conditions, we are ready to abandon the concept of individuality. Thus the lack of freedom is especially clear in the hypnotized person, or in the person who, after having been hypnotized and then awakened, carries out the commands given to him during hypnosis; others around him recognize his lack of freedom, although he is not aware of it himself. A lack of freedom is shown also by a sleeping person, or by one who is under the influence of certain drugs, which change his thoughts, emotions and behavior; also by the insane. The thoughts of individuals suffering from the same type of mental disease may be very similar in character and only slightly or hardly at all individualized. A further evidence of automatism is furnished by cases of a split or double personality, where normally connecting memories are conspicuously disconnected at a certain point, although in reality every individual has a multiple personality dependent on the existence within him of mutually incompatible suggestions,

attribute to his psychical individuality the character of uniqueness to a much higher degree than it actually possesses. As we have stated, also the psychical individuality (personality) is a mosaic in which the constituents were acquired from various sources, partly as inheritance of peculiarities in the structure and function of certain organs from the ancestors of the individual, partly through suggestions and thoughts taken over from other individuals with whom he has been in contact. Very little, as a rule, has he himself contributed to this mosaic. What distinguishes an individual is the way in which these various constituents are combined and accentuated. The uniqueness of the psychical individuality is furthermore due to the uniqueness of individual experiences. And here again, the individual experiences are not really unique, but the series as a whole, the order in which they are joined together and the relative significance of each one of these experiences for the individual may be unique. The psychical individuality represents, thus, a biological-historical system, in which environmental factors play a very important role. It is the selection and chronological order as well as the intensity of the influences and experiences which have acted on the individual, especially in the course of his most formative, impressionable period of life—but also later—which help to determine his psychical character and his uniqueness. But even these historical factors are not usually entirely unique. Other individuals have experiences, if not identical, at least somewhat similar, and the scientific analysis of the effects of these series of experiences on the nature of the psychical individuality seems feasible. In contrast to the psychical individuality which thus represents a biological-historical system, the other two types of individualities, one of them based on the character of the various tissues and organs and of their combinations, of which in certain respects the psychical individuality really represents merely a part, and the other based on the character of the individuality differential, are purely biological, and much more independent of more or less accidental environmental factors and more fixed and determined in their nature. This genetic fixity and relative independence of accidental conditions characterizes especially the individuality differentials, but almost equally as much the tissue and organ differentials.

3. *The permanence of our individuality.* We are involved in a struggle with nature, which we learn to dominate only within a very limited range. Our organism ages, becomes sick and dies. This natural struggle invariably ends in defeat. But the directing, apparently self-determining agent in our individuality, that which seems really characteristic of us, we conceive as imperishable, eternal. We have built thought structures expressing and justifying this interpretation. But even if we do not accept these views, still we live essentially in the world of our thoughts, emotions and wishes, which are "we," and these thoughts we feel are free, not limited by the realities of life and nature. And in this thought-world we apparently continue to act quite independently of the changes which actually take place in us, of our real fate. Thus we see ourselves as continuing to live after our death in the world of our thoughts. We want to transmit to the world our thoughts and attitudes and change the world into one more suited to our needs, into a better world,

acter. On the other hand, we often attribute to the outside and we blame others for what is essentially determined by our own inherited and acquired constitutions.

Our mental processes, and in particular the thoughts concerned with ourselves, function in a definite mental milieu, in a medium of nerve and endocrine gland activity, to which we are accommodated. We have adapted ourselves to a certain intensity of feeling, energy or lassitude, to a certain kind of emotional reaction, to a certain mode of thinking and rhythm of reactions taking place within ourselves and within others. In this milieu we feel at home; it is here that we are accustomed to direct our thoughts, our movements, to talk and to respond to other persons. If these processes take place smoothly, we do not especially become aware of ourselves, of the control we exert over ourselves and we take the continuity of our personality for granted. We do not usually notice very gradual and connected changes. But if our milieu is abruptly, acutely changed, our reactions are changed in a sudden way also; thus, under unfavorable environmental conditions, under the influence of drugs, in sickness, we may become tense, irritable, involved in conflict with others and with ourselves. Our usually self-controlling thoughts cannot at once accommodate themselves to the altered organism on which they have to act; they find different effects, different responses; there is an interference with our personality, a disturbance in our continuity, a rift within us.

Similarly we are accustomed to the set of suggestions in which we live. Often insidious in their action, these function in a mild way because we are adapted to them, because they have been with us for a long time. They are not considered as strange to us, as an outside product forced on us, but as something adopted by us, or as having originated in our own thought system; they arouse no sensation of discontinuity in our self-directing personality. But if we receive a sudden command, then we react to it as an interference and as opposed to us. This changed situation is no longer compatible with our feeling of freedom, continuity and self-consistency. The same result follows if forced thoughts, new to us, incompatible with the rest of our personality, develop in us. Then the idea of continuity in our individuality is interrupted; we experience an interference with our individuality, especially if under the changed circumstances our responses become different and uncontrolled. However, should other disturbing factors interfere also with our ability to reason, to analyze, then the consciousness of cleavage and of discontinuity in our personality may be lacking.

But under normal conditions we have the feeling that we are constant in a changing world. We have an intimate acquaintance with our environment, we have the knowledge of what to expect in it; there is a certain permanence from day to day in our bodily organism and it is distinct from other organisms. Thus we are satisfied that our individuality is continuous, forming one definite entity, that there is identity of the self in one moment with the self of the past and of the future.

And furthermore, the individual himself, as well as those around him,

attributes, beliefs and principles, occupations and professions, family associations, or other personal relationships such as friendships and feuds. It is essentially as members of such groups that we enter into communication with individuals; we possess the group-suggestions and we may be subject to acutely acting ones, such as those manifest, for instance, in the mob spirit, and individuals are largely, to us, therefore, representatives of groups, symbols of various activities, tendencies, principles or associations of human beings. Yet within these groups individuals are distinguished by the possession of special group characteristics. Each individual is a composite as a member of many groups and these groupings are not the same in different individuals. But in addition, we recognize various distinctive signs of individuals, such as structural characteristics, movements, ways of speaking, expressions of various thoughts and emotions and special attitudes which distinguish one individual from another and which are partly independent of groups.

However it is, after all, only a small part of the individuality of others and of himself which each person learns to know. The meaning of individuality, therefore, is based largely on the subjective experiences of the individual himself, and the knowledge thus derived is imperfect and faulty. The recognition of the distinctive features of the individual and of the meaning of individuality are problems with which the study of the body and mind is concerned. Science provides instruments for the analysis of the physical and psychical mosaic of which the individual is composed and makes possible the investigation of genetic and environmental factors entering into his constitution. But science in carrying out this analysis splits individuality into many constituent parts, which then are joined together again into new groups or types more significant than the conventional ones which a more superficial observation furnishes. Science thus shows that what is most significant in individuals as separate entities is not the elements of which the individual consists, but the mode and the quantitative manner in which these elements are joined together, and in this sense it deprives to some extent individuality of its distinctiveness and uniqueness and it diminishes in man what has been considered as the most characteristic feature of his individuality.

As we have pointed out earlier in this chapter there is a far going difference between the psychical-social and the physical-physiological individuality. In contrast to the physiological and physical individuality which is distinct and sharply separated from the surrounding world, the psychical individuality forms in certain respects one connected whole with its environment. The evidence given in our preceding discussion has shown that in the psychical sphere the individual is not sharply separated from the non living things and other organisms. The psychical individuality is composed of elements which are interwoven in such an intricate way with the world surrounding the individual that it is difficult to make a sharp distinction between those elements which belong to the one and to the other. Moreover the intricacy of these connections increases the difficulty of establishing in the actions of the individual the relations of cause and effect. If we consider the fact that the psychical-social individuality depends largely on the nervous system for its

the idea of which means something to us although we are no longer here to experience it, to benefit by it. Our children shall live in our spirit and continue in our ways and lead our efforts to fruition. But even in the face of death men also keep up their petty ambitions and competitive self assertion. The individual has lived and may still continue to live in a thought-world, which does not take heed of his waning powers nor of the mortal disease which may affect him; these changes often do not tend to enter as real constituents into the construction of his mind. His thought-world may remain fixed and he does not foresee an end to it.

4. *Self-justification of our individuality.* The individual lives in a struggle with nature and with his social world; in this struggle he receives injuries and inflicts injuries. In others, he sees himself and the injury of others he feels as his own injury. There exist laws which are disregarded by him and he acts contrary to them; he suffers from the pangs of conscience and fears the consequences of what he does. In such a conflict he needs approval of his actions and his individuality, he needs justification for his existence, absolution for his failures and for his infringements of those laws which are believed to be absolute. And yet, in determining his responsibility, he often attributes to himself what originated in others, and to his environment he attributes what was his own; even here he is unable to discern the real from the unreal and, insofar, he again lives in a world of illusions.

However, all these psychical reactions in human beings which have here been discussed and which tend to express and safeguard their individuality, are not elementary psychical phenomena, but are conditioned by the social setting in which they occur, by the social traditions, customs, and ethical standards which direct and control the life of the social groups, large or small, to which the individuals belong. The psychical individuality as we have just described it, exists therefore only in an advanced stage of human social development, where the sets of active suggestions are wider, more numerous and more varied than in the more primitive societies, but where they are also less firmly fixed, more accessible to influences which may change them, where the manifestations of the social struggle are more complex and may affect also the thought-life and emotions of the individual to a higher degree, and above all, where a social reservoir of scientific and philosophical thought is available, which may serve as a source of inner psychical goods to which the individual has access. There has thus taken place an evolution also of the psychical-social individuality; but it is the task of the history of civilization to trace this evolution.

Individuals are the units which constitute groups. Groups of various kinds are aggregations of individuals in which the distinctive characters of component parts are disregarded and characteristics common to all are used to distinguish one group from all the other groups. In a certain sense, the group concepts are thus opposed to and destructive of the features which constitute the individual. The group concepts as far as they concern man are abstractions. This applies to all groups, whether nations, races, economic classes, social castes, or societies of various kinds; also, whether they are based on moral

Chapter 3

The Evolution of Individuality

In a preceding part we have followed the evolution of organisms from their primitive beginnings to man. At first, the organisms are relatively simple as far as the differentiation of their organs and the character of their organismal differentials are concerned. They are still very plastic, responding readily with a modification of organ and tissue formation to certain changes in the environment. They also reproduce with ease lost parts of their body, even relatively small pieces having the power to do so. Presumably this relatively great plasticity of the organs and the relative simplicity of the organismal differentials are connected with each other.

With advancing evolution, the plasticity of the organism, its readiness to respond to the environment, decreases; more and more the organism becomes a fixed, closed system, in which structural complexity and integration increase; at the same time, the organs become more specialized and the organismal differentials more differentiated and individualized. The increasing independence of the environment applies not only to the adult organism, but also to the embryo, whose development, in the higher organisms, takes place within the body of the adult mother; in this way the influence of environmental factors on development is more completely excluded. Within the mother's body the greater specialization of the organ and organismal differentials takes place, the finer structural differentiation and the fuller integration occur and a more individualized organism is formed. It is born in a state in which the animal is more or less fully developed as far as its structural characteristics are concerned.

Concurrently and intimately connected with this increase in the specialization of organ and organismal differentials and in the individualization of the organism, a greater refinement in the immune mechanisms is established. This latter change adds still further to the individualization of the organism and tends to transfer a greater part of its reaction to certain environmental alterations from the external world into the interior of the animal. We can consider the gradual refinement of the organismal differentials and of the processes on which their manifestation depends, as well as the increasing delicacy and significance of the immunity reactions, which are largely based on corresponding changes in the various differentials, as mechanisms of defense on the part of the organism as a whole against interference from the outside, and therefore as mechanisms guaranteeing the integrity of the organism and its increased independence of the environment. As a result of these alterations, there is a change in the circuit of relationship between organism and environment, in that the influence of the environment on the organism becomes less and the effect of the organism on the environment becomes greater in the course of evolution. With this increasing refinement of

development and expression and that the nervous system acts as the agent and representative of the outer world within us it can be readily understood that there is an intimate connection between the psychical individuality and the outer world.

It is these relations between the individuality and the surrounding world as well as the relations which exist between individuality in the physical and physiological sense (body) and in the psychical sense (soul) which have been the main problems with which philosophy has dealt throughout its history. Therefore essentially the problems of philosophy have been largely concerned with the meaning of individuality.

Thoughts reproduced environment, and in the environment thus reproduced the social environment became more and more prominent; this type of interaction between organism and environment did not, therefore, take place in the same rigid way as in primitive organisms, such as insects, but with the creation of thought so many possibilities of response arose that the actions of the individual became very varied. At the same time the environment affected the organism in a new way through the development of imagination and suggestion.

As a result of these modifications, the apparent freedom and the greater individualization in the psychical-social sense of the higher organism have evolved. In this second circuit the environment influences and in a delicate manner changes the living substratum on which it acts; it gains in importance in comparison with the inheritable rigidity of the basic functions of the first circuit. While thus in the more primitive organism genetic conditions determine more directly the behavior, and while also in the highest, most complex organism, man, the basic functions are essentially fixed in a rigid way by genetic factors, there develops in man a special mechanism which makes possible a very sensitive interaction between organism and environment; in this sphere, the environment becomes a factor of great importance in directing the behavior of the individual. In the more primitive organisms, individuality is largely fixed; in man the psychical individuality is to a great extent modifiable, environmental in character. The content of our mind is given us by the daily experiences in life; in particular, by the suggestions of the persons we meet, in whom all these influences have also entered; but it is also given us by poets, artists, philosophers and scientists. In this psychical-social aspect our individuality has become the more modifiable, the greater the refinement of the nervous system has become.

The factors entering into human behavior have reached such a degree of complexity that the actions of individuals are often unpredictable, and the illusion of indeterminateness in willing and doing arises. Furthermore, the contacts between individual and environment have become not only much more varied and extensive—the individual being in contact with an ever enlarging part of the universe—but they are also intensified Suffering and pain of the mind and elations have evolved, which had previously not existed. With the increase in the importance of the central nervous mechanism there increased the anticipations, the dread of disease and suffering and of annihilation, as well as the fear of the intentions and actions of other human beings; but there developed also new satisfactions of wider visions, of deeper understanding. The human organism is not only shaped by the environment, but more and more it reacts against it and learns to understand and modify it. There develops the pleasure of creative, playful interaction with the environment; but not only does man interact with the environment, he interacts and learns to experiment with and, to a certain extent, to shape his own psychical-social organism and those of others. While thus, in certain respects, the individuality becomes increasingly pronounced, in other respects, in consequence of the more and more intricate interaction between environment and

individualization there decreases the potentiality of small somatic parts to reproduce the whole organism in an apparently unending series, and correspondingly, there decreases the potentiality to immortal life of these parts. Instead, this potentiality to immortality becomes dependent entirely on the special mechanism of sexual reproduction, which in the higher organism is of such a kind that at no time are the sex cells exposed to the direct action of the environment. However, certain somatic cells and tissues of these differentiated organisms still retain their potential immortality, as exemplified in the propagation of tumor tissues in succeeding generations of hosts and of embryonal tissues kept in tissue culture; but in these cases the tissues are able to manifest a potential immortality only if they are supplied with an environment in which the complex substances specifically needed for their growth, as well as other needs, are experimentally provided. They cannot be propagated in the natural inorganic environment, which would be adequate for the propagation of parts of lower organisms.

In this first circuit of the relationship between organism and environment, the evolution of individuality consisted in the development of an organism which became more and more autonomous, more and more independent of the environment, except that it needed the environment as the source of its food and energy. However, there arose on the basis of and closely connected with this circuit, a second one, in which the evolution went in the opposite direction. Here, the increasing complexity and differentiation of the organism led, on the contrary, to a more intimate interaction between the organism and the environment. This environment became increasingly important and it determined to a large extent the fate of the individual, his ability to maintain himself and to find satisfaction in his world. In this second circuit, organism and environment were connected by way of sense organs, nervous system and muscular system, by means of which the organism acted again on the environment. The evolution of this circuit depended on the refinement of these specific organs and their organ differentials. Thus the organism came into contact with a much more extensive part of its environment and the contacts became more specialized and variegated. An early stage of this development was reached with the production of conditioned reflexes in the interaction between environment and organism. Alterations occurring in the nervous system as the result of repeated stimulation, made it possible that simple environmental factors more or less loosely or accidently connected with the direct stimulus, were able effectively to replace the latter. Furthermore, pictures and thoughts, representing environmental factors and systems of such factors, eventually could substitute for the environmental factors and systems themselves and thus determine the mode of reaction of the organism. Thus, conditioned thought reflexes developed. Concomitantly, a great refinement took place in the manner in which the nervous system was affected by these outer and inner factors, and the intracerebral reactions became longer-lasting and more significant. These various changes left important after-effects in the form of memory and thoughts; analysis and synthesis thus became possible.

in the form of words and are transmitted from generation to generation. They are used as suggestions and give origin to conditioned thought reflexes, which are associated with a certain environment. This system of thought reflexes, with the accompanying emotions and the psychical-social environment, forms then, one whole; it carries injuries and pain. From this reservoir in general, we receive our instruments in the social struggle; we may leave unchanged what we have taken and give it back again with all its inherent imperfections; or we may add new imperfections by using concepts faultily in the social struggle. Only gradually and very slowly is a modification of the thought-reservoir accomplished through the psychical reactions of individuals who suffer, and these may find expression in the work of poets, philosophers and scientists; also through the play of mind which leads to the creation of new ideas.

However, the social thought-reservoir acquires an additional significance for us. In seeking for something to take the place of the absolute, yet something to which we may fix our aims and motives and which provides more than a satisfaction of our passing needs, something which is lasting and independent of the changing and ephemeral in us and in things around us, we turn to this thought-reservoir in our search for a constant in the universe and in man; we attempt to convert it into a trustworthy source of our valuations and principles and, therefore, also of our inner psychical goods. To build it up, make it consistent, to extend it, so that it becomes more and more universal in the course of time, we conceive as our highest task. In these efforts there begins to develop, step by step, a common, general reservoir for all humanity, instead of the many particularistic group reservoirs which had originally existed.

Our psychical-social individuality, representing combinations of thoughts, wishes and wills, accompanied by emotions and functioning within the framework of the body—the elementary organ systems—with the aid of the nervous system and of the system of hormones, represents thus something intermediate between our bodily organism and the social thought-reservoir. It takes its origin in the body and reaches out into this reservoir, which is common to all but with which we each have our individualized contacts. Within this reservoir is that which is relatively constant, but constant only as compared with the fleeting existence of the individual. The individual varies and disintegrates, but our social thought-reservoir appears lasting, the depository of fixed values. Here is what remains of the individual, what he took from it and what he added to it. The psychical-social individual to a large extent consists of things borrowed from this reservoir, and to it, in the pain of the natural and social struggle, he joins his fortunes. Here he deposits his discoveries, thoughts and principles, to which, if possible, he adds his individual name, so that in the reservoir he may live when his physical self has died; at the same time in so doing he eliminates his individuality as much as he can from the social struggle and disappears in the impersonal, the unselfish, in the realm of lasting principles where all individual pain and individual desires end.

Thus a fourth and shorter circuit has developed as the latest phase in the

psychical-social individuality, a separation between individuality and environment, especially the social environment, becomes impossible. Hence the second circuit has been refined into a modified, a third circuit, which leads from the social as well as the natural environment to the nervous system, to thoughts and suggestions, and back again to the social and natural environment.

In this third circuit thoughts and suggestions have been profoundly modified, not only by the natural but also by the social environment, and, moreover, they have become the more important and powerful, because they did not live an isolated, separate existence, but were connected with systems of traditions, myths, philosophy and science. All of these latter formed one whole, a system acting as a huge social thought-reservoir, which became more and more independent of the individual. However, the individual not only received from this thought-reservoir, thoughts and suggestions determining his actions and orientation to world and life, but conversely, he contributed to it his own thoughts, suggestions and emotional reactions, as manifested in the various forms of art, in science and philosophy and the conventions of social life. Intensified satisfactions were felt in the creation of concepts concerning the universe and in victories won in the social and natural struggle, and these concepts entered into the social thought-reservoir and thus became the possession of all, freed from the index of the individuality which had contributed to their creation and which was able to create because it had previously received important constituents from this common source.

At the same time this social thought-reservoir has become the source of much suffering because of its mode of origin, reflecting as it does our imperfect manner of thinking. Reality, the totality of our environment in its interaction with our body and thoughts, is too vast and too complex for us; it is more than we can manipulate. We can concentrate at one time only on certain features of it; necessarily we abstract and, subsequently, parts which diverse abstractions have in common are synthesized by us into a new concept. Thus generalization follows abstraction. Some of these procedures are carried out in a relatively satisfactory manner, such as the abstractions and generalizations in mathematics and science; and also the more simple abstractions used in ordinary life, sensations such as hot, cold, red, blue; or comparisons of quantities of weighable substances: "much," "little," these all are fairly satisfactory abstractions, serviceable and more or less in harmony with reality. But there are many inadequate or false and arbitrary abstractions and generalizations. They occur especially in all those realms of life where our emotions are affected, and where the social struggle enters. This is true especially of many moral, political and social concepts, such as those expressing approval or condemnation, those of fashions and rituals; the fact that they are often purely arbitrary, and not representative of real and *significant* things and processes is not usually recognized. And some of these concepts not only represent inadequate abstractions and generalizations, but also injurious ones; this applies in particular to many social concepts which serve as instruments in the social struggle for material and distinctive psychical goods. All these ideas enter the psychical-social reservoir; here they remain, as it were, frozen

evolution of the psychical individuality: (1) The most primitive stage is represented by that of the simple reflex mechanisms, to which the simple conditioned reflex is added as an important extension. The action of hormones may further complicate this mechanism. (2) Superimposed upon this stage is the one in which there are active more or less isolated, disconnected pictures of things and events, developing in response to the needs of the moment; they may become memories and may direct actions. (3) In a third stage, thoughts which represent simple abstractions are produced. These may exert their effects as suggestions, extending from others to ourselves; or as auto-suggestions originating within us. It is partly, or perhaps largely, by means of auto-suggestions that our thought determine our present and our intended, our future, actions and attitudes. (4) The highest stage is reached with the functioning of extended, conscious and rational thoughts which then may affect our actions and attitudes. The further development of the psychical individuality coincides with the history of civilization. In the evolution from the first to subsequent stages the directness of the relationship between the organism and the environment decreases; more and more there are placed between the two, psychical factors; and, concomitantly, the contact with and the understanding of the environment enlarges and deepens. Thus, in the interaction between our psychical individuality and the outer world, constituents of the latter play a greatly predominating role, so that the relative importance of external factors and of inner factors in the functioning of the organism becomes entirely different. The essential content of what we call "mind" is composed of things given us from the outside, from the non-living environment, and, above all, from the living, social, human environment. While the simple reflex action is largely of the same kind in all individuals of the same species, with increasing psychical development and especially with the development of analytic thought, the differentiation between individual organism is greatly increased and real psychical individualities are created.

But while the dependence of our personality on the environment, and especially on the social environment, becomes greater, at the same time consistency of thought and the building up of a social thought-reservoir, with which we enter into increasingly intimate connection, cause our individuality to become more fixed, steady, independent of the environment, which, on the contrary, we now begin to modify and to shape more and more in accordance with our desires; and this environment, on which we are able to act and which we can alter, includes in certain respects our own organism, in the bodily as well as in the psychical-social sense.

These various circuits, which develop as steps in the evolution of individuality, remain connected with one another in a more or less intimate manner. The later circuits are superimposed upon and depend for their existence and function upon the primary circuit, which is the basic one. While the latter gives thus to the more complex, higher circuits the possibility of maintenance, development and of further evolution, while changes occurring in the primary circuit affect all the higher circuits, and while, in particular, its derangement causes serious interference with the function of the higher circuits, there

evolution of individuality; it connects the individual with the social thought-reservoir and from there leads back to the individual. These relations of the individual to the thought-reservoir were used by man in an attempt to regain the potential immortality which, in the sphere of the first circuit, had been lost with advancing evolution, and thus to obtain compensation for the injuries and destruction experienced in the social and natural struggle. But this effort is in vain. The thought-reservoir reflects the world, the social environment, life as a whole, and in making connections with it a part of the psychical individuality is sacrificed. This last circuit represents the highest point, the last phase in the evolution of individuality, the latter entering into that which is common to all and thus in part giving up its separate existence.

Yet, while the individual lives and struggles, the social thought-reservoir exerts a real function in his activities. It has a steadying, stabilizing effect on him and it may restrict the excesses in which his personality may express itself. Thus he is limited, is made less free, but at the same time it renders the individual, in his sensory-nervous-muscular circuit, less dependent on the environment. It brings continuity into his reactions, which are then determined not solely by momentary impressions and responses, but by thoughts and traditions acting as relative constants, as principles in an ever changing world and life. In this manner a development is achieved in the psychical-social sphere, not unlike that acquired in the first, the primary circuit, which latter results in the building up of a very differentiated system, more and more detached from and independent of the environment, a process characterized by such conditions as homoiothermia, homoiohydria, homoiotonia, and, in general, by what has been called by Cannon, homoiostasis. Corresponding to this latter development, there has resulted from the evolution of the thought-reservoir and from its interaction with the individual a kind of psychical homoiostasis, in which the psychical individuality is weighted down, anchored and fixed to something that holds it firm in the movements and struggles of existence.

There has thus taken place an evolution of two types of individuality. The first is connected with the differentiation of the organ differentials and with the evolution of the individuality differential and its manifestations, from a very primitive character to the state of great refinement reached in mammals. The second is connected with the evolution of the psychical-social factors, leading to the gradual creation and refinement of the indivdual in the psychical-social sense. This second evolutionary process is related only indirectly to the development of the individuality differentials; it depends directly upon the increasing complexity and refinement of certain organ differentials, especially of the nervous system. There is, therefore, no perfect parallelism between these two evolutionary processes. While in the first process a gradual, step-by-step development of the individuality differential occurs, in the second process the most important, far-reaching change has taken place suddenly in the transition from anthropoid apes to man.

Corresponding in certain respects to the types of circuits which connect the individual and his environment four stages may be distinguished in the

the furthergoing analysis of man and his life, while it may provide satisfaction and in the end give strength and calmness, may also under certain conditions, interfere with the normal reflex and instinctive processes; especially if it tends to reproduce events in the painful social struggle, these pictures may have disturbing effects and be injurious to the thinking individual. Thinking in general causes fatigue, especially consistent thinking that subordinates itself to reality which it wishes to express, and it is a difficult process. It is due to this fact that in general man avoids analytic, objective thinking as much as possible and devotes himself rather to the processes of willing and doing, and to emotional experiences, and the events in the social struggle are allowed to take their course and the serious consequences of this struggle may become aggravated.

Evolution has thus led to a gradual loss of the plasticity and to an increasing differentiation, integration, rigidity and fixity of the body, and associated with this process there has developed an increasing individualization by various means. This development has taken place, (1) by a refinement of the organismal differentials and the creation of the individuality differentials or by making the latter manifest; (2) by an increasing differentiation and integration of the organ and tissue systems, and (3) by the creation and intensification of the psychical individuality with the aid of certain organ systems. Associated with this increase in individualization and close integration, deficiencies have developed in the organization of the body which become more apparent with advancing age and in the end lead to the death of the individual.

Not only the bodily organization but also the psychical individuality which has developed in the course of evolution is imperfect and deficient. This is composed largely of suggestions which exist as separate, mutually disharmonious constituents of the mind, whereas the integrating true thought processes, which would be able to unify these disconnected parts into one consistent whole and to effect greater harmony between the individual and human society, do not function adequately.

Parallel to the evolution of the individual, the social life as well has undergone a progressive evolution. It began with the rigidity of the social organization of animal groups, as represented by the relations between certain unicellular organisms, by primitive colonies, by the essentially fixed and determined character of insect societies and by the less firmly knit social organizations among vertebrates. Within the vertebrates a further evolution in the same direction has taken place; it made its greatest advance in the change from the social life of monkeys and apes to that of human beings. In the latter, free imagination and thought even in the restricted way in which they are active have almost completely overcome the limitations of animal societies. Human society is thus no longer fixed, but it has become a modifiable state determined by varied suggestions as well as by rational thought and directed by the needs and desires for material and psychical goods. While the simple and distinctive psychical goods used and valued by human beings also may have roots in the psychical life of animal groups, they

takes place also the reverse interaction, inasmuch as the character and functioning of the higher circuits in which thoughts more and more predominate, affects very potently the character and normal function of the primary circuit.

As a result of this evolution of individuality, suffering, injuries, pains and satisfactions are multiplied, intensified and individualized; and all these experiences in the psychical-social sphere affect also the primary individuality as manifested in the first circuit, the effects of psychical experience becoming very far-reaching and important for the organism as a whole. More and more, psychical experiences come to depend on intricate social organizations, on social structures, in which the social struggle, the creation, acquisition and distribution not only of material but also of simple and distinctive psychical goods and the state of inner psychical goods play an important part. Thus, with the increasing differentiation and refinement of the sense organs and of the central nervous system and with the corresponding development of a complex psychical and social life, our interactions with the environment are extended, our experiences multiplied and our living intensified. The psychical individuality which has now been created, attempts to maintain and to elevate itself and in these efforts it collides with similar efforts of other individuals and this is one of the principal causes of the social struggle which greatly affects the psychical life and may lead to injuries. Under these conditions there develop the need and desire for an adequate environment, suitable for bodily and psychical requirements; the individual is spurred on to modify the natural and social environment and the social thought-reservoir, and by these means to effect changes also in the character of the material as well as of the various types of psychical goods, and so to gain rest and security for himself in the natural and social struggle.

It is primarily by facing directly the difficulties and dangers in the social and natural struggle, by analysing and learning to understand these difficulties, that he may hope to overcome them and be victorious in these struggles as far as this is possible. Thus he may in the end achieve for himself calmness and strength and he will give to others understanding. The product of analytic and generalized thinking has thus entered the social thought reservoir; it has become an instrument which man uses and which may be of advantage to him in the material and social struggle. This advantage is now accessible to all and is no longer individual, but it is enduring only, if the underlying thinking process was sound.

Others may renounce the life of the social and natural struggle, as far as their thought is concerned; they know the impossibility of actually overcoming the struggle in life and they retire into a type of thinking in which thought is freed more and more from the disturbing elements inherent in the sphere of the social struggle. Thought reproduces events, life and world instead of serving as an instrument in the social struggle and it also enters the social thought reservoir. Man by means of his thoughts divests himself in part of his psychical individuality and identifies himself with the whole.

But, as indicated already, thinking, especially when it is concerned with

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have undergone a much farther and individualized development in man, in whom the inner psychical goods have been entirely newly created. This evolution has led to the abolishment of those rigid modes of organization, which characterize the different types of animal societies, and has replaced these by the modifiable constitution of human society which is accessible to direction by rational thought.

To recapitulate, the evolution of individuality has taken place in two opposite directions. The body developed from a state of relative variability, which depended upon and was to a large extent directed by the environment, to a state of relative fixity, autonomy and unyieldingness, much less subject to environmental conditions. From the point of view of the bodily organism, the inner constitutional factors have overbalanced therefore the environmental factors to a larger extent in the further advanced organism than in the more primitive ones. There then took place, parallel to the evolution of the body, the evolution of the sense organs, of the central nervous system and of the psychical-social mechanism, in which the environment again has become of increasing significance. Associated with these two tendencies in evolution there occurred the development of the social struggle as a manifestation of the greater importance of psychical activity and psychical needs, in contrast to the natural struggle, which was primarily concerned with the satisfaction of the requirements of the body.

Thus, in matters which relate to man as a psychical-social organism, it is the environment which has become a preponderating influence and which largely determines his fate. To adapt the psychical-social environment to the needs of man, so that he can function in the most adequate manner, is, therefore, the most important task which humanity has now to face.

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THIS BOOK

THE

BIOLOGICAL BASIS

OF

INDIVIDUALITY

A Second Printing

By LEO LOEB

was set and bound by The Collegiate Press of Menasha, Wisconsin, and lithoprinted by Edwards Brothers, Inc., of Ann Arbor, Michigan. The type face is Linotype Old Style No. 1 set 10 point on 12 point. The type page is 28 x 48 picas. The text paper is 50-lb. White Offset